

e) Expression of P788P in *E. Coli*

A truncated, N-terminal portion, of P788P (residues 1-644 of SEQ ID NO: 777; referred to as P788P-N) fused with a C-terminal 6xHis Tag was expressed in *E. coli* as follows. P788P cDNA was amplified using the primers AW080 and AW081 (SEQ ID NO: 672 and 673). AW080 is a sense cloning primer with an NdeI site. AW081 is an antisense cloning primer with a XhoI site. The PCR-amplified P788P, as well as the vector pCRX1, were digested with NdeI and XhoI. Vector and insert were ligated and transformed into NovaBlue cells. Colonies were randomly screened for insert and then sequenced. P788P-N clone #6 was confirmed to be identical to the designed construct. The expression construct P788P-N #6/pCRX1 was transformed into *E. coli* BL21 CodonPlus-RIL competent cells. After induction, most of the cells grew well, achieving OD600 of greater than 2.0 after 3 hr. Coomassie stained SDS-PAGE showed an over-expressed band at about 75 kD. Western blot analysis using a 6xHisTag antibody confirmed the band was P788P-N. The determined cDNA sequence for P788P-N is provided in SEQ ID NO: 674, with the corresponding amino acid sequence being provided in SEQ ID NO: 675.

f) Expression of P510S in *E. Coli*

The P510S protein has 9 potential transmembrane domains and is predicted to be located at the plasma membrane. The C-terminal protein of this protein, as well as the predicted third extracellular domain of P510S were expressed in *E. coli* as follows.

The expression construct referred to as Ra12-P501S-C was designed to have a 6 HisTag at the N-terminal end, followed by the *M. tuberculosis* antigen Ra12 (SEQ ID NO: 676) and then the C-terminal portion of P510S (amino residues 1176-1261 of SEQ ID NO: 538). Full-length P510S was used to amplify the P510S-C fragment by PCR using the primers AW056 and AW057 (SEQ ID NO: 677 and 678, respectively). AW056 is a sense cloning primer with an EcoRI site. AW057 is an antisense primer with stop and XhoI sites. The amplified P501S fragment and Ra12/pCRX1 were digested with EcoRI and XhoI and then purified. The insert and

vector were ligated together and transformed into NovaBlue. Colonies were randomly screened for insert and sequences. For protein expression, the expression construct was transformed into *E. coli* BL21 (DE3) CodonPlus-RIL competent cells. A mini-induction screen was performed to optimize the expression conditions. After induction the cells grew well, achieving OD 600 nm greater than 2.0 after 3 hours. Coomassie stain SDS-PAGE showed a highly over-expressed band at approx. 30 kD. Though this is higher than the expected molecular weight, western blot analysis was positive, showing this band to be the His tag-containing protein. The optimized culture conditions are as follows. Dilute overnight culture/daytime culture (LB + kanamycin + chloramphenicol) into 2xYT (with kanamycin and chloramphenicol) at a ratio of 25 ml culture to 1 liter 2xYT. Allow to grow at 37 °C until OD600 = 0.6. Take an aliquot out as T0 sample. Add 1 mM IPTG and allow to grow at 30 °C for 3 hours. Take out a T3 sample, spin down cells and store at -80 °C. The determined cDNA and amino acid sequences for the Ra12-P510S-C construct are provided in SEQ ID NO: 679 and 682, respectively.

The expression construct P510S-C was designed to have a 5' added start codon and a glycine (GGA) codon and then the P510S C terminal fragment followed by the in frame 6x histidine tag and stop codon from the pET28b vector. The cloning strategy is similar to that used for Ra12-P510S-C, except that the PCR primers employed were those shown in SEQ ID NO: 685 and 686, respectively and the NcoI/XhoI cut in pET28b was used. The primer of SEQ ID NO: 685 created a 5' NcoI site and added a start codon. The antisense primer of SEQ ID NO: 686 creates a XhoI site on P510S C terminal fragment. Clones were confirmed by sequencing. For protein expression, the expression construct was transformed into *E. coli* BL21 (DE3) CodonPlus-RIL competent cells. An OD600 of greater than 2.0 was obtained 30 hours after induction. Coomassie stained SDS-PAGE showed an over-expressed band at about 11 kD. Western blot analysis confirmed that the band was P510S-C, as did N-terminal protein sequencing. The optimized culture conditions are as follows: dilute overnight culture/daytime culture (LB + kanamycin + chloramphenicol) into 2x YT (+ kanamycin and chloramphenicol) at a ratio of 25 mL culture to 1 liter 2x YT, and allow to grow at

37 °C until an OD 600 of about 0.5 is reached. Take out an aliquot as T0 sample. Add 1 mM IPTG and allow to grow at 30 °C for 3 hours. Spin down the cells and store at -80 °C until purification. The determined cDNA and amino acid sequences for the P510S-C construct are shown in SEQ ID NO: 680 and 683, respectively.

- 5 The predicted third extracellular domain of P510S (P510S-E3; residues 328-676 of SEQ ID NO: 538) was expressed in *E. coli* as follows. The P510S fragment was amplified by PCR using the primers shown in SEQ ID NO: 687 and 688. The primer of SEQ ID NO: 687 is a sense primer with an NdeI site for use in ligating into pPDM. The primer of SEQ ID NO: 688 is an antisense primer with an added XhoI site
- 10 for use in ligating into pPDM. The resulting fragment was cloned to pPDM at the NdeI and XhoI sites. Clones were confirmed by sequencing. For protein expression, the clone was transformed into *E. coli* BL21 (DE3) CodonPlus-RIL competent cells. After induction, an OD600 of greater than 2.0 was achieved after 3 hours. Coomassie stained SDS-PAGE showed an over-expressed band at about 39 kD, and N-terminal sequencing
- 15 confirmed the N-terminal to be that of P510S-E3. Optimized culture conditions are as follows: dilute overnight culture/daytime culture (LB + kanamycin + chloramphenicol) into 2x YT (kanamycin and chloramphenicol) at a ratio of 25 ml culture to 1 liter 2x YT. Allow to grow at 37 °C until OD 600 equals 0.6. Take out an aliquot as T0 sample. Add 1 mM IPTG and allow to grow at 30 °C for 3 hours. Take out a T3
- 20 sample, spin down the cells and store at -80 °C until purification. The determined cDNA and amino acid sequences for the P501S-E3 construct are provided in SEQ ID NO: 681 and 684, respectively.

g) Expression of P775S in *E. Coli*

- 25 The antigen P775P contains multiple open reading frames (ORF). The third ORF, encoding the protein of SEQ ID NO: 483, has the best motif score. An expression fusion construct containing the *M. tuberculosis* antigen Ra12 (SEQ ID NO: 676) and P775P-ORF3 with an N-terminal 6x HisTag was prepared as follows. P775P-ORF3 was amplified using the sense PCR primers of SEQ ID NO: 689 and the anti-sense PCR primer of SEQ ID NO: 690. The PCR amplified fragment of P775P and

Ra12/pCRX1 were digested with the restriction enzymes EcoRI and XhoI. Vector and insert were ligated and then transformed into NovaBlue cells. Colonies were randomly screened for insert and then sequenced. A clone having the desired sequence was transformed into *E. coli* BL21 (DE3) CodonPlus-RIL competent cells. Two hours after
5 induction, the cell density peaked at OD600 of approximately 1.8. Coomassie stained SDS-PAGE showed an over-expressed band at about 31 kD. Western blot using 6x HisTag antibody confirmed that the band was Ra12-P775P-ORF3. The determined cDNA and amino acid sequences for the fusion construct are provided in SEQ ID NO: 691 and 692, respectively.

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H) EXPRESSION OF A P703P HIS TAG FUSION PROTEIN IN *E. COLI*

The cDNA for the coding region of P703P was prepared by PCR using the primers of SEQ ID NO: 693 and 694. The PCR product was digested with EcoRI restriction enzyme, gel purified and cloned into a modified pET28 vector with a His tag
15 in frame, which had been digested with Eco72I and EcoRI restriction enzymes. The correct construct was confirmed by DNA sequence analysis and then transformed into *E. coli* BL21 (DE3) pLys S expression host cells. The determined amino acid and cDNA sequences for the expressed recombinant P703P are provided in SEQ ID NO: 695 and 696, respectively.

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I) EXPRESSION OF A P705P HIS TAG FUSION PROTEIN IN *E. COLI*

The cDNA for the coding region of P705P was prepared by PCR using the primers of SEQ ID NO: 697 and 698. The PCR product was digested with EcoRI restriction enzyme, gel purified and cloned into a modified pET28 vector with a His tag
25 in frame, which had been digested with Eco72I and EcoRI restriction enzymes. The correct construct was confirmed by DNA sequence analysis and then transformed into *E. coli* BL21 (DE3) pLys S and BL21 (DE3) CodonPlus expression host cells. The determined amino acid and cDNA sequences for the expressed recombinant P705P are provided in SEQ ID NO: 699 and 700, respectively.

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J) EXPRESSION OF A P711P HIS TAG FUSION PROTEIN IN E. COLI

The cDNA for the coding region of P711P was prepared by PCR using the primers of SEQ ID NO: 701 and 702. The PCR product was digested with EcoRI restriction enzyme, gel purified and cloned into a modified pET28 vector with a His tag in frame, which had been digested with Eco721 and EcoRI restriction enzymes. The correct construct was confirmed by DNA sequence analysis and then transformed into *E. coli* BL21 (DE3) pLys S and BL21 (DE3) CodonPlus expression host cells. The determined amino acid and cDNA sequences for the expressed recombinant P711P are provided in SEQ ID NO: 703 and 704, respectively.

EXAMPLE 18

PREPARATION AND CHARACTERIZATION OF ANTIBODIES AGAINST PROSTATE-SPECIFIC POLYPEPTIDES

a) Preparation and Characterization of Polyclonal Antibodies against P703P, P504S and P509S

Polyclonal antibodies against P703P, P504S and P509S were prepared as follows.

Each prostate tumor antigen expressed in an *E. coli* recombinant expression system was grown overnight in LB broth with the appropriate antibiotics at 37°C in a shaking incubator. The next morning, 10 ml of the overnight culture was added to 500 ml to 2x YT plus appropriate antibiotics in a 2L-baffled Erlenmeyer flask. When the Optical Density (at 560 nm) of the culture reached 0.4-0.6, the cells were induced with IPTG (1 mM). Four hours after induction with IPTG, the cells were harvested by centrifugation. The cells were then washed with phosphate buffered saline and centrifuged again. The supernatant was discarded and the cells were either frozen for future use or immediately processed. Twenty ml of lysis buffer was added to the cell pellets and vortexed. To break open the *E. coli* cells, this mixture was then run

through the French Press at a pressure of 16,000 psi. The cells were then centrifuged again and the supernatant and pellet were checked by SDS-PAGE for the partitioning of the recombinant protein. For proteins that localized to the cell pellet, the pellet was resuspended in 10 mM Tris pH 8.0, 1% CHAPS and the inclusion body pellet was washed and centrifuged again. This procedure was repeated twice more. The washed inclusion body pellet was solubilized with either 8 M urea or 6 M guanidine HCl containing 10 mM Tris pH 8.0 plus 10 mM imidazole. The solubilized protein was added to 5 ml of nickel-chelate resin (Qiagen) and incubated for 45 min to 1 hour at room temperature with continuous agitation. After incubation, the resin and protein mixture were poured through a disposable column and the flow through was collected. The column was then washed with 10-20 column volumes of the solubilization buffer. The antigen was then eluted from the column using 8M urea, 10 mM Tris pH 8.0 and 300 mM imidazole and collected in 3 ml fractions. A SDS-PAGE gel was run to determine which fractions to pool for further purification.

As a final purification step, a strong anion exchange resin such as HiPrepQ (Biorad) was equilibrated with the appropriate buffer and the pooled fractions from above were loaded onto the column. Each antigen was eluted off the column with a increasing salt gradient. Fractions were collected as the column was run and another SDS-PAGE gel was run to determine which fractions from the column to pool. The pooled fractions were dialyzed against 10 mM Tris pH 8.0. The proteins were then vialled after filtration through a 0.22 micron filter and the antigens were frozen until needed for immunization.

Four hundred micrograms of each prostate antigen was combined with 100 micrograms of muramyl dipeptide (MDP). Every four weeks rabbits were boosted with 100 micrograms mixed with an equal volume of Incomplete Freund's Adjuvant (IFA). Seven days following each boost, the animal was bled. Sera was generated by incubating the blood at 4°C for 12-4 hours followed by centrifugation.

Ninety-six well plates were coated with antigen by incubating with 50 microliters (typically 1 microgram) of recombinant protein at 4 °C for 20 hours. 250 microliters of BSA blocking buffer was added to the wells and incubated at room

temperature for 2 hours. Plates were washed 6 times with PBS/0.01% Tween. Rabbit sera was diluted in PBS. Fifty microliters of diluted sera was added to each well and incubated at room temperature for 30 min. Plates were washed as described above before 50 microliters of goat anti-rabbit horse radish peroxidase (HRP) at a 1:10000 dilution was added and incubated at room temperature for 30 min. Plates were again washed as described above and 100 microliters of TMB microwell peroxidase substrate was added to each well. Following a 15 min incubation in the dark at room temperature, the colorimetric reaction was stopped with 100 microliters of 1N H₂SO₄ and read immediately at 450 nm. All polyclonal antibodies showed immunoreactivity to the appropriate antigen.

b) Preparation and Characterization of Antibodies against P501S

A murine monoclonal antibody directed against the carboxy-terminus of the prostate-specific antigen P501S was prepared as follows.

A truncated fragment of P501S (amino acids 355-526 of SEQ ID NO: 113) was generated and cloned into the pET28b vector (Novagen) and expressed in *E. coli* as a thioredoxin fusion protein with a histidine tag. The trx-P501S fusion protein was purified by nickel chromatography, digested with thrombin to remove the trx fragment and further purified by an acid precipitation procedure followed by reverse phase HPLC.

Mice were immunized with truncated P501S protein. Serum bleeds from mice that potentially contained anti-P501S polyclonal sera were tested for P501S-specific reactivity using ELISA assays with purified P501S and trx-P501S proteins. Serum bleeds that appeared to react specifically with P501S were then screened for P501S reactivity by Western analysis. Mice that contained a P501S-specific antibody component were sacrificed and spleen cells were used to generate anti-P501S antibody producing hybridomas using standard techniques. Hybridoma supernatants were tested for P501S-specific reactivity initially by ELISA, and subsequently by FACS analysis of reactivity with P501S transduced cells. Based on these results, a monoclonal hybridoma referred to as 10E3 was chosen for further subcloning. A number of subclones were

generated, tested for specific reactivity to P501S using ELISA and typed for IgG isotype. The results of this analysis are shown below in Table V. Of the 16 subclones tested, the monoclonal antibody 10E3-G4-D3 was selected for further study.

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Table V

Isotype analysis of murine anti-P501S monoclonal antibodies

| Hybridoma clone | Isotype | Estimated [Ig] in supernatant ($\mu\text{g/ml}$) |
|-----------------|---------|--|
| 4D11 | IgG1 | 14.6 |
| 1G1 | IgG1 | 0.6 |
| 4F6 | IgG1 | 72 |
| 4H5 | IgG1 | 13.8 |
| 4H5-E12 | IgG1 | 10.7 |
| 4H5-EH2 | IgG1 | 9.2 |
| 4H5-H2-A10 | IgG1 | 10 |
| 4H5-H2-A3 | IgG1 | 12.8 |
| 4H5-H2-A10-G6 | IgG1 | 13.6 |
| 4H5-H2-B11 | IgG1 | 12.3 |
| 10E3 | IgG2a | 3.4 |
| 10E3-D4 | IgG2a | 3.8 |
| 10E3-D4-G3 | IgG2a | 9.5 |
| 10E3-D4-G6 | IgG2a | 10.4 |
| 10E3-E7 | IgG2a | 6.5 |
| 8H12 | IgG2a | 0.6 |

The specificity of 10E3-G4-D3 for P501S was examined by FACS analysis. Specifically, cells were fixed (2% formaldehyde, 10 minutes), permeabilized (0.1% saponin, 10 minutes) and stained with 10E3-G4-D3 at 0.5 – 1 $\mu\text{g/ml}$, followed by incubation with a secondary, FITC-conjugated goat anti-mouse Ig antibody (Pharmingen, San Diego, CA). Cells were then analyzed for FITC fluorescence using an Excalibur fluorescence activated cell sorter. For FACS analysis of transduced cells, B-LCL were retrovirally transduced with P501S. For analysis of infected cells, B-LCL were infected with a vaccinia vector that expresses P501S. To demonstrate specificity in these assays, B-LCL transduced with a different antigen (P703P) and uninfected B-LCL vectors were utilized. 10E3-G4-D3 was shown to bind with P501S-transduced B-

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LCL and also with P501S-infected B-LCL, but not with either uninfected cells or P703P-transduced cells.

To determine whether the epitope recognized by 10E3-G4-D3 was found on the surface or in an intracellular compartment of cells, B-LCL were transduced with
5 P501S or HLA-B8' as a control antigen and either fixed and permeabilized as described above or directly stained with 10E3-G4-D3 and analyzed as above. Specific recognition of P501S by 10E3-G4-D3 was found to require permeabilization, suggesting that the epitope recognized by this antibody is intracellular.

The reactivity of 10E3-G4-D3 with the three prostate tumor cell lines
10 Lncap, PC-3 and DU-145, which are known to express high, medium and very low levels of P501S, respectively, was examined by permeabilizing the cells and treating them as described above. Higher reactivity of 10E3-G4-D3 was seen with Lncap than with PC-3, which in turn showed higher reactivity than DU-145. These results are in agreement with the real time PCR and demonstrate that the antibody specifically
15 recognizes P501S in these tumor cell lines and that the epitope recognized in prostate tumor cell lines is also intracellular.

Specificity of 10E3-G4-D3 for P501S was also demonstrated by Western blot analysis. Lysates from the prostate tumor cell lines Lncap, DU-145 and PC-3, from P501S-transiently transfected HEK293 cells, and from non-transfected HEK293 cells
20 were generated. Western blot analysis of these lysates with 10E3-G4-D3 revealed a 46 kDa immunoreactive band in Lncap, PC-3 and P501S-transfected HEK cells, but not in DU-145 cells or non-transfected HEK293 cells. P501S mRNA expression is consistent with these results since semi-quantitative PCR analysis revealed that P501S mRNA is expressed in Lncap, to a lesser but detectable level in PC-3 and not at all in DU-145
25 cells. Bacterially expressed and purified recombinant P501S (referred to as P501SStr2) was recognized by 10E3-G4-D3 (24 kDa), as was full-length P501S that was transiently expressed in HEK293 cells using either the expression vector VR1012 or pCEP4. Although the predicted molecular weight of P501S is 60.5 kDa, both transfected and "native" P501S run at a slightly lower mobility due to its hydrophobic nature.

Immunohistochemical analysis was performed on prostate tumor and a panel of normal tissue sections (prostate, adrenal, breast, cervix, colon, duodenum, gall bladder, ileum, kidney, ovary, pancreas, parotid gland, skeletal muscle, spleen and testis). Tissue samples were fixed in formalin solution for 24 hours and embedded in paraffin before being sliced into 10 micron sections. Tissue sections were permeabilized and incubated with 10E3-G4-D3 antibody for 1 hr. HRP-labeled anti-mouse followed by incubation with DAB chromogen was used to visualize P501S immunoreactivity. P501S was found to be highly expressed in both normal prostate and prostate tumor tissue but was not detected in any of the other tissues tested.

To identify the epitope recognized by 10E3-G4-D3, an epitope mapping approach was pursued. A series of 13 overlapping 20-21 mers (5 amino acid overlap; SEQ ID NO: 489-501) was synthesized that spanned the fragment of P501S used to generate 10E3-G4-D3. Flat bottom 96 well microtiter plates were coated with either the peptides or the P501S fragment used to immunize mice, at 1 microgram/ml for 2 hours at 37 °C. Wells were then aspirated and blocked with phosphate buffered saline containing 1% (w/v) BSA for 2 hours at room temperature, and subsequently washed in PBS containing 0.1% Tween 20 (PBST). Purified antibody 10E3-G4-D3 was added at 2 fold dilutions (1000 ng – 16 ng) in PBST and incubated for 30 minutes at room temperature. This was followed by washing 6 times with PBST and subsequently incubating with HRP-conjugated donkey anti-mouse IgG (H+L) Affinipure F(ab') fragment (Jackson ImmunoResearch, West Grove, PA) at 1:20000 for 30 minutes. Plates were then washed and incubated for 15 minutes in tetramethyl benzidine. Reactions were stopped by the addition of 1N sulfuric acid and plates were read at 450 nm using an ELISA plate reader. As shown in Fig. 8, reactivity was seen with the peptide of SEQ ID NO: 496 (corresponding to amino acids 439-459 of P501S) and with the P501S fragment but not with the remaining peptides, demonstrating that the epitope recognized by 10E3-G4-D3 is localized to amino acids 439-459 of SEQ ID NO: 113.

In order to further evaluate the tissue specificity of P501S, multi-array immunohistochemical analysis was performed on approximately 4700 different human tissues encompassing all the major normal organs as well as neoplasias derived from

these tissues. Sixty-five of these human tissue samples were of prostate origin. Tissue sections 0.6 mm in diameter were formalin-fixed and paraffin embedded. Samples were pretreated with HIER using 10 mM citrate buffer pH 6.0 and boiling for 10 min. Sections were stained with 10E3-G4-D3 and P501S immunoreactivity was visualized with HRP. All the 65 prostate tissues samples (5 normal, 55 untreated prostate tumors, 5 hormone refractory prostate tumors) were positive, showing distinct perinuclear staining. All other tissues examined were negative for P501S expression.

c) Preparation and Characterization of Antibodies against P503S

- 10 A fragment of P503S (amino acids 113-241 of SEQ ID NO: 114) was expressed and purified from bacteria essentially as described above for P501S and used to immunize both rabbits and mice. Mouse monoclonal antibodies were isolated using standard hybridoma technology as described above. Rabbit monoclonal antibodies were isolated using Selected Lymphocyte Antibody Method (SLAM) technology at
- 15 Immgenics Pharmaceuticals (Vancouver, BC, Canada). Table VI, below, lists the monoclonal antibodies that were developed against P503S.

Table VI

| Antibody | Species |
|----------|---------|
| 20D4 | Rabbit |
| JA1 | Rabbit |
| 1A4 | Mouse |
| 1C3 | Mouse |
| 1C9 | Mouse |
| 1D12 | Mouse |
| 2A11 | Mouse |
| 2H9 | Mouse |
| 4H7 | Mouse |
| 8A8 | Mouse |
| 8D10 | Mouse |
| 9C12 | Mouse |
| 6D12 | Mouse |

The DNA sequences encoding the complementarity determining regions (CDRs) for the rabbit monoclonal antibodies 20D4 and JA1 were determined and are provided in SEQ ID NO: 502 and 503, respectively.

5 In order to better define the epitope binding region of each of the antibodies, a series of overlapping peptides were generated that span amino acids 109-213 of SEQ ID NO: 114. These peptides were used to epitope map the anti-P503S monoclonal antibodies by ELISA as follows. The recombinant fragment of P503S that was employed as the immunogen was used as a positive control. Ninety-six well
10 microtiter plates were coated with either peptide or recombinant antigen at 20 ng/well overnight at 4 °C. Plates were aspirated and blocked with phosphate buffered saline containing 1% (w/v) BSA for 2 hours at room temperature then washed in PBS containing 0.1% Tween 20 (PBST). Purified rabbit monoclonal antibodies diluted in PBST were added to the wells and incubated for 30 min at room temperature. This was
15 followed by washing 6 times with PBST and incubation with Protein-A HRP conjugate at a 1:2000 dilution for a further 30 min. Plates were washed six times in PBST and incubated with tetramethylbenzidine (TMB) substrate for a further 15 min. The reaction was stopped by the addition of 1N sulfuric acid and plates were read at 450 nm using an ELISA plate reader. ELISA with the mouse monoclonal antibodies was performed with
20 supernatants from tissue culture run neat in the assay.

All of the antibodies bound to the recombinant P503S fragment, with the exception of the negative control SP2 supernatant. 20D4, JA1 and 1D12 bound strictly to peptide #2101 (SEQ ID NO: 504), which corresponds to amino acids 151-169 of SEQ ID NO: 114. 1C3 bound to peptide #2102 (SEQ ID NO: 505), which corresponds
25 to amino acids 165-184 of SEQ ID NO: 114. 9C12 bound to peptide #2099 (SEQ ID NO: 522), which corresponds to amino acids 120-139 of SEQ ID NO: 114. The other antibodies bind to regions that were not examined in these studies.

Subsequent to epitope mapping, the antibodies were tested by FACS analysis on a cell line that stably expressed P503S to confirm that the antibodies bind to
30 cell surface epitopes. Cells stably transfected with a control plasmid were employed as

a negative control. Cells were stained live with no fixative. 0.5 ug of anti-P503S monoclonal antibody was added and cells were incubated on ice for 30 min before being washed twice and incubated with a FITC-labelled goat anti-rabbit or mouse secondary antibody for 20 min. After being washed twice, cells were analyzed with an Excalibur
5 fluorescent activated cell sorter. The monoclonal antibodies 1C3, 1D12, 9C12, 20D4 and JA1, but not 8D3, were found to bind to a cell surface epitope of P503S.

In order to determine which tissues express P503S, immunohistochemical analysis was performed, essentially as described above, on a panel of normal tissues (prostate, adrenal, breast, cervix, colon, duodenum, gall bladder,
10 ileum, kidney, ovary, pancreas, parotid gland, skeletal muscle, spleen and testis). HRP-labeled anti-mouse or anti-rabbit antibody followed by incubation with TMB was used to visualize P503S immunoreactivity. P503S was found to be highly expressed in prostate tissue, with lower levels of expression being observed in cervix, colon, ileum and kidney, and no expression being observed in adrenal, breast, duodenum, gall
15 bladder, ovary, pancreas, parotid gland, skeletal muscle, spleen and testis.

Western blot analysis was used to characterize anti-P503S monoclonal antibody specificity. SDS-PAGE was performed on recombinant (rec) P503S expressed in and purified from bacteria and on lysates from HEK293 cells transfected with full length P503S. Protein was transferred to nitrocellulose and then Western blotted with
20 each of the anti-P503S monoclonal antibodies (20D4, JA1, 1D12, 6D12 and 9C12) at an antibody concentration of 1 ug/ml. Protein was detected using horse radish peroxidase (HRP) conjugated to either a goat anti-mouse monoclonal antibody or to protein A-sepharose. The monoclonal antibody 20D4 detected the appropriate molecular weight 14 kDa recombinant P503S (amino acids 113-241) and the 23.5 kDa
25 species in the HEK293 cell lysates transfected with full length P503S. Other anti-P503S monoclonal antibodies displayed similar specificity by Western blot.

d) Preparation and Characterization of Antibodies against P703P

Rabbits were immunized with either a truncated (P703Ptl; SEQ ID NO: 172) or full-length mature form (P703Pfl; SEQ ID NO: 523) of recombinant P703P

protein was expressed in and purified from bacteria as described above. Affinity purified polyclonal antibody was generated using immunogen P703Pfl or P703Ptrl attached to a solid support. Rabbit monoclonal antibodies were isolated using SLAM technology at Immgenics Pharmaceuticals. Table VII below lists both the polyclonal and monoclonal antibodies that were generated against P703P.

Table VII

| Antibody | Immunogen | Species/type |
|--|-----------|-------------------|
| Aff. Purif. P703P (truncated); #2594 | P703Ptrl | Rabbit polyclonal |
| Aff. Purif. P703P (full length); #9245 | P703Pfl | Rabbit polyclonal |
| 2D4 | P703Ptrl | Rabbit monoclonal |
| 8H2 | P703Ptrl | Rabbit monoclonal |
| 7H8 | P703Ptrl | Rabbit monoclonal |

The DNA sequences encoding the complementarity determining regions (CDRs) for the rabbit monoclonal antibodies 8H2, 7H8 and 2D4 were determined and are provided in SEQ ID NO: 506-508, respectively.

Epitope mapping studies were performed as described above. Monoclonal antibodies 2D4 and 7H8 were found to specifically bind to the peptides of SEQ ID NO: 509 (corresponding to amino acids 145-159 of SEQ ID NO: 172) and SEQ ID NO: 510 (corresponding to amino acids 11-25 of SEQ ID NO: 172), respectively. The polyclonal antibody 2594 was found to bind to the peptides of SEQ ID NO: 511-514, with the polyclonal antibody 9427 binding to the peptides of SEQ ID NO: 515-517.

The specificity of the anti-P703P antibodies was determined by Western blot analysis as follows. SDS-PAGE was performed on (1) bacterially expressed recombinant antigen; (2) lysates of HEK293 cells and Ltk^{-/-} cells either untransfected or transfected with a plasmid expressing full length P703P; and (3) supernatant isolated from these cell cultures. Protein was transferred to nitrocellulose and then Western blotted using the anti-P703P polyclonal antibody #2594 at an antibody concentration of 1 µg/ml. Protein was detected using horse radish peroxidase (HRP) conjugated to an anti-rabbit antibody. A 35 kDa immunoreactive band could be observed with

recombinant P703P. Recombinant P703P runs at a slightly higher molecular weight since it is epitope tagged. In lysates and supernatants from cells transfected with full length P703P, a 30 kDa band corresponding to P703P was observed. To assure specificity, lysates from HEK293 cells stably transfected with a control plasmid were also tested and were negative for P703P expression. Other anti-P703P antibodies showed similar results.

Immunohistochemical studies were performed as described above, using anti-P703P monoclonal antibody. P703P was found to be expressed at high levels in normal prostate and prostate tumor tissue but was not detectable in all other tissues tested (breast tumor, lung tumor and normal kidney).

e) Preparation and Characterization of Antibodies against P504S

Full-length P504S (SEQ ID NO: 108) was expressed and purified from bacteria essentially as described above for P501S and employed to raise rabbit monoclonal antibodies using Selected Lymphocyte Antibody Method (SLAM) technology at Immgenics Pharmaceuticals (Vancouver, BC, Canada). The anti-P504S monoclonal antibody 13H4 was shown by Western blot to bind to both expressed recombinant P504S and to native P504S in tumor cells.

Immunohistochemical studies using 13H4 to assess P504S expression in various prostate tissues were performed as described above. A total of 104 cases, including 65 cases of radical prostatectomies with prostate cancer (PC), 26 cases of prostate biopsies and 13 cases of benign prostate hyperplasia (BPH), were stained with the anti-P504S monoclonal antibody 13H4. P504S showed strongly cytoplasmic granular staining in 64/65 (98.5%) of PCs in prostatectomies and 26/26 (100%) of PCs in prostatic biopsies. P504S was stained strongly and diffusely in carcinomas (4+ in 91.2% of cases of PC; 3+ in 5.5%; 2+ in 2.2% and 1+ in 1.1%) and high grade prostatic intraepithelial neoplasia (4+ in all cases). The expression of P504S did not vary with Gleason score. Only 17/91 (18.7%) of cases of NP/BPH around PC and 2/13 (15.4%) of BPH cases were focally (1+, no 2+ to 4+ in all cases) and weakly positive for P504S in large glands. Expression of P504S was not found in small atrophic glands, postatrophic hyperplasia, basal cell hyperplasia and transitional cell metaplasia in either biopsies or

prostatectomies. P504S was thus found to be over-expressed in all Gleason scores of prostate cancer (98.5 to 100% of sensitivity) and exhibited only focal positivities in large normal glands in 19/104 of cases (82.3% of specificity). These findings indicate that P504S may be usefully employed for the diagnosis of prostate cancer.

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EXAMPLE 19

CHARACTERIZATION OF CELL SURFACE EXPRESSION AND
CHROMOSOME LOCALIZATION OF THE PROSTATE-SPECIFIC ANTIGEN P501S

10 This example describes studies demonstrating that the prostate-specific antigen P501S is expressed on the surface of cells, together with studies to determine the probable chromosomal location of P501S.

The protein P501S (SEQ ID NO: 113) is predicted to have 11 transmembrane domains. Based on the discovery that the epitope recognized by the anti-
15 P501S monoclonal antibody 10E3-G4-D3 (described above in Example 17) is intracellular, it was predicted that following transmembrane determinants would allow the prediction of extracellular domains of P501S. Fig. 9 is a schematic representation of the P501S protein showing the predicted location of the transmembrane domains and the intracellular epitope described in Example 17. Underlined sequence represents the
20 predicted transmembrane domains, bold sequence represents the predicted extracellular domains, and italicized sequence represents the predicted intracellular domains. Sequence that is both bold and underlined represents sequence employed to generate polyclonal rabbit serum. The location of the transmembrane domains was predicted using HHMTOP as described by Tusnady and Simon (Principles Governing Amino
25 Acid Composition of Integral Membrane Proteins: Applications to Topology Prediction, *J. Mol. Biol.* 283:489-506, 1998).

Based on Fig. 9, the P501S domain flanked by the transmembrane domains corresponding to amino acids 274-295 and 323-342 is predicted to be extracellular. The peptide of SEQ ID NO: 518 corresponds to amino acids 306-320 of
30 P501S and lies in the predicted extracellular domain. The peptide of SEQ ID NO: 519,

which is identical to the peptide of SEQ ID NO: 518 with the exception of the substitution of the histidine with an asparagine, was synthesized as described above. A Cys-Gly was added to the C-terminus of the peptide to facilitate conjugation to the carrier protein. Cleavage of the peptide from the solid support was carried out using the following cleavage mixture: trifluoroacetic acid:ethanediol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for two hours, the peptide was precipitated in cold ether. The peptide pellet was then dissolved in 10% v/v acetic acid and lyophilized prior to purification by C18 reverse phase hplc. A gradient of 5-60% acetonitrile (containing 0.05% TFA) in water (containing 0.05% TFA) was used to elute the peptide. The purity of the peptide was verified by hplc and mass spectrometry, and was determined to be >95%. The purified peptide was used to generate rabbit polyclonal antisera as described above.

Surface expression of P501S was examined by FACS analysis. Cells were stained with the polyclonal anti-P501S peptide serum at 10 µg/ml, washed, incubated with a secondary FITC-conjugated goat anti-rabbit Ig antibody (ICN), washed and analyzed for FITC fluorescence using an Excalibur fluorescence activated cell sorter. For FACS analysis of transduced cells, B-LCL were retrovirally transduced with P501S. To demonstrate specificity in these assays, B-LCL transduced with an irrelevant antigen (P703P) or nontransduced were stained in parallel. For FACS analysis of prostate tumor cell lines, Lncap, PC-3 and DU-145 were utilized. Prostate tumor cell lines were dissociated from tissue culture plates using cell dissociation medium and stained as above. All samples were treated with propidium iodide (PI) prior to FACS analysis, and data was obtained from PI-excluding (i.e., intact and non-permeabilized) cells. The rabbit polyclonal serum generated against the peptide of SEQ ID NO: 519 was shown to specifically recognize the surface of cells transduced to express P501S, demonstrating that the epitope recognized by the polyclonal serum is extracellular.

To determine biochemically if P501S is expressed on the cell surface, peripheral membranes from Lncap cells were isolated and subjected to Western blot analysis. Specifically, Lncap cells were lysed using a dounce homogenizer in 5 ml of homogenization buffer (250 mM sucrose, 10 mM HEPES, 1mM EDTA, pH 8.0, 1

complete protease inhibitor tablet (Boehringer Mannheim)). Lysate samples were spun at 1000 g for 5 min at 4 °C. The supernatant was then spun at 8000g for 10 min at 4 °C. Supernatant from the 8000g spin was recovered and subjected to a 100,000g spin for 30 min at 4 °C to recover peripheral membrane. Samples were then separated by SDS-PAGE and Western blotted with the mouse monoclonal antibody 10E3-G4-D3 (described above in Example 17) using conditions described above. Recombinant purified P501S, as well as HEK293 cells transfected with and over-expressing P501S were included as positive controls for P501S detection. LCL cell lysate was included as a negative control. P501S could be detected in Lncap total cell lysate, the 8000g (internal membrane) fraction and also in the 100,000g (plasma membrane) fraction. These results indicate that P501S is expressed at, and localizes to, the peripheral membrane.

To demonstrate that the rabbit polyclonal antiserum generated to the peptide of SEQ ID NO: 519 specifically recognizes this peptide as well as the corresponding native peptide of SEQ ID NO: 518, ELISA analyses were performed. For these analyses, flat-bottomed 96 well microtiter plates were coated with either the peptide of SEQ ID NO: 519, the longer peptide of SEQ ID NO: 520 that spans the entire predicted extracellular domain, the peptide of SEQ ID NO: 521 which represents the epitope recognized by the P501S-specific antibody 10E3-G4-D3, or a P501S fragment (corresponding to amino acids 355-526 of SEQ ID NO: 113) that does not include the immunizing peptide sequence, at 1 µg/ml for 2 hours at 37 °C. Wells were aspirated, blocked with phosphate buffered saline containing 1% (w/v) BSA for 2 hours at room temperature and subsequently washed in PBS containing 0.1% Tween 20 (PBST). Purified anti-P501S polyclonal rabbit serum was added at 2 fold dilutions (1000 ng - 125 ng) in PBST and incubated for 30 min at room temperature. This was followed by washing 6 times with PBST and incubating with HRP-conjugated goat anti-rabbit IgG (H+L) Affinipure F(ab') fragment at 1:20000 for 30 min. Plates were then washed and incubated for 15 min in tetramethyl-benzidine. Reactions were stopped by the addition of 1N sulfuric acid and plates were read at 450 nm using an ELISA plate reader. As shown in Fig. 11, the anti-P501S polyclonal rabbit serum specifically recognized the

peptide of SEQ ID NO: 519 used in the immunization as well as the longer peptide of SEQ ID NO: 520, but did not recognize the irrelevant P501S-derived peptides and fragments.

In further studies, rabbits were immunized with peptides derived from the P501S sequence and predicted to be either extracellular or intracellular, as shown in Fig. 9. Polyclonal rabbit sera were isolated and polyclonal antibodies in the serum were purified, as described above. To determine specific reactivity with P501S, FACS analysis was employed, utilizing either B-LCL transduced with P501S or the irrelevant antigen P703P, of B-LCL infected with vaccinia virus-expressing P501S. For surface expression, dead and non-intact cells were excluded from the analysis as described above. For intracellular staining, cells were fixed and permeabilized as described above. Rabbit polyclonal serum generated against the peptide of SEQ ID NO: 548, which corresponds to amino acids 181-198 of P501S, was found to recognize a surface epitope of P501S. Rabbit polyclonal serum generated against the peptide SEQ ID NO: 551, which corresponds to amino acids 543-553 of P501S, was found to recognize an epitope that was either potentially extracellular or intracellular since in different experiments intact or permeabilized cells were recognized by the polyclonal sera. Based on similar deductive reasoning, the sequences of SEQ ID NO: 541-547, 549 and 550, which correspond to amino acids 109-122, 539-553, 509-520, 37-54, 342-359, 295-323, 217-274, 143-160 and 75-88, respectively, of P501S, can be considered to be potential surface epitopes of P501S recognized by antibodies.

In further studies, mouse monoclonal antibodies were raised against amino acids 296 to 322 to P501S, which are predicted to be in an extracellular domain. A/J mice were immunized with P501S/adenovirus, followed by subsequent boosts with an *E. coli* recombinant protein, referred to as P501N, that contains amino acids 296 to 322 of P501S, and with peptide 296-322 (SEQ ID NO: 755) coupled with KLH. The mice were subsequently used for splenic B cell fusions to generate anti-peptide hybridomas. The resulting 3 clones, referred to as 4F4 (IgG1,kappa), 4G5 (IgG2a,kappa) and 9B9 (IgG1,kappa), were grown for antibody production. The 4G5 mAb was purified by passing the supernatant over a Protein A-sepharose column,

followed by antibody elution using 0.2M glycine, pH 2.3. Purified antibody was neutralized by the addition of 1M Tris, pH 8, and buffer exchanged into PBS.

For ELISA analysis, 96 well plates were coated with P501S peptide 296-322 (referred to as P501-long), an irrelevant P775 peptide, P501S-N, P501TR2, P501S-long-KLH, P501S peptide 306-319 (referred to as P501-short)-KLH, or the irrelevant peptide 2073-KLH, all at a concentration of 2 ug/ml and allowed to incubate for 60 minutes at 37 °C. After coating, plates were washed 5X with PBS + 0.1% Tween and then blocked with PBS, 0.5% BSA, 0.4% Tween20 for 2 hours at room temperature. Following the addition of supernatants or purified mAb, the plates were incubated for 60 minutes at room temperature. Plates were washed as above and donkey anti-mouse IgHRP-linked secondary antibody was added and incubated for 30 minutes at room temperature, followed by a final washing as above. TMB peroxidase substrate was added and incubated 15 minutes at room temperature in the dark. The reaction was stopped by the addition of 1N H₂SO₄ and the OD was read at 450 nM. All three hybrid clones secreted mAb that recognized peptide 296-322 and the recombinant protein P501N.

For FACS analysis, HEK293 cells were transiently transfected with a P501S/VR1012 expression constructs using Fugene 6 reagent. After 2 days of culture, cells were harvested and washed, then incubated with purified 4G5 mAb for 30 minutes on ice. After several washes in PBS, 0.5% BSA, 0.01% azide, goat anti-mouse Ig-FITC was added to the cells and incubated for 30 minutes on ice. Cells were washed and resuspended in wash buffer including 1% propidium iodide and subjected to FACS analysis. The FACS analysis confirmed that amino acids 296-322 of P501S are in an extracellular domain and are cell surface expressed.

The chromosomal location of P501S was determined using the GeneBridge 4 Radiation Hybrid panel (Research Genetics). The PCR primers of SEQ ID NO: 528 and 529 were employed in PCR with DNA pools from the hybrid panel according to the manufacturer's directions. After 38 cycles of amplification, the reaction products were separated on a 1.2% agarose gel, and the results were analyzed through the Whitehead Institute/MIT Center for Genome Research web server

(<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmappper.pl>) to determine the probable chromosomal location. Using this approach, P501S was mapped to the long arm of chromosome 1 at WI-9641 between q32 and q42. This region of chromosome 1 has been linked to prostate cancer susceptibility in hereditary prostate cancer (Smith *et al.* 5 *Science* 274:1371-1374, 1996 and Berthon *et al. Am. J. Hum. Genet.* 62:1416-1424, 1998). These results suggest that P501S may play a role in prostate cancer malignancy.

EXAMPLE 20

REGULATION OF EXPRESSION OF THE PROSTATE-SPECIFIC ANTIGEN P501S

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Steroid (androgen) hormone modulation is a common treatment modality in prostate cancer. The expression of a number of prostate tissue-specific antigens have previously been demonstrated to respond to androgen. The responsiveness of the prostate-specific antigen P501S to androgen treatment was examined in a tissue culture 15 system as follows.

Cells from the prostate tumor cell line LNCaP were plated at 1.5×10^6 cells/T75 flask (for RNA isolation) or 3×10^5 cells/well of a 6-well plate (for FACS analysis) and grown overnight in RPMI 1640 media containing 10% charcoal-stripped fetal calf serum (BRL Life Technologies, Gaithersburg, MD). Cell culture was 20 continued for an additional 72 hours in RPMI 1640 media containing 10% charcoal-stripped fetal calf serum, with 1 nM of the synthetic androgen Methyltrienolone (R1881; New England Nuclear) added at various time points. Cells were then harvested for RNA isolation and FACS analysis at 0, 1, 2, 4, 8, 16, 24, 28 and 72-hours post androgen addition. FACS analysis was performed using the anti-P501S antibody 10E3- 25 G4-D3 and permeabilized cells.

For Northern analysis, 5-10 micrograms of total RNA was run on a formaldehyde denaturing gel, transferred to Hybond-N nylon membrane (Amersham Pharmacia Biotech, Piscataway, NJ), cross-linked and stained with methylene blue. The filter was then prehybridized with Church's Buffer (250 mM Na_2HPO_4 , 70 mM H_3PO_4 , 30 1 mM EDTA, 1% SDS, 1% BSA in pH 7.2) at 65 °C for 1 hour. P501S DNA was

labeled with ^{32}P using High Prime random-primed DNA labeling kit (Boehringer Mannheim). Unincorporated label was removed using MicroSpin S300-HR columns (Amersham Pharmacia Biotech). The RNA filter was then hybridized with fresh Church's Buffer containing labeled cDNA overnight, washed with 1X SCP (0.1 M NaCl, 0.03 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 M Na_2EDTA), 1% sarkosyl (n-lauroylsarcosine) and exposed to X-ray film.

Using both FACS and Northern analysis, P501S message and protein levels were found to increase in response to androgen treatment.

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EXAMPLE 20

PREPARATION OF FUSION PROTEINS OF PROSTATE-SPECIFIC ANTIGENS

The example describes the preparation of a fusion protein of the prostate-specific antigen P703P and a truncated form of the known prostate antigen PSA. The truncated form of PSA has a 21 amino acid deletion around the active serine site. The expression construct for the fusion protein also has a restriction site at 3' end, immediately prior to the termination codon, to aid in adding cDNA for additional antigens.

The full-length cDNA for PSA was obtained by RT-PCR from a pool of RNA from human prostate tumor tissues using the primers of SEQ ID NO: 607 and 608, and cloned in the vector pCR-Blunt II-TOPO. The resulting cDNA was employed as a template to make two different fragments of PSA by PCR with two sets of primers (SEQ ID NO: 609 and 610; and SEQ ID NO: 611 and 612). The PCR products having the expected size were used as templates to make truncated forms of PSA by PCR with the primers of SEQ ID NO: 611 and 613, which generated PSA (delta 208-218 in amino acids). The cDNA for the mature form of P703P with a 6X histidine tag at the 5' end, was prepared by PCR with P703P and the primers of SEQ ID NO: 614 and 615. The cDNA for the fusion of P703P with the truncated form of PSA (referred to as FOPP) was then obtained by PCR using the modified P703P cDNA and the truncated form of PSA cDNA as templates and the primers of SEQ ID NO: 614 and 615. The FOPP

cDNA was cloned into the NdeI site and XhoI site of the expression vector pCRX1, and confirmed by DNA sequencing. The determined cDNA sequence for the fusion construct FOPP is provided in SEQ ID NO: 616, with the amino acid sequence being provided in SEQ ID NO: 617.

- 5 The fusion FOPP was expressed as a single recombinant protein in *E. coli* as follows. The expression plasmid pCRX1FOPP was transformed into the *E. coli* strain BL21-CodonPlus RIL. The transformant was shown to express FOPP protein upon induction with 1 mM IPTG. The culture of the corresponding expression clone was inoculated into 25 ml LB broth containing 50 ug/ml kanamycin and 34 ug/ml
- 10 chloramphenicol, grown at 37 °C to OD600 of about 1, and stored at 4 °C overnight. The culture was diluted into 1 liter of TB LB containing 50 ug/ml kanamycin and 34 ug/ml chloramphenicol, and grown at 37 °C to OD600 of 0.4. IPTG was added to a final concentration of 1 mM, and the culture was incubated at 30 °C for 3 hours. The cells were pelleted by centrifugation at 5,000 RPM for 8 min. To purify the protein, the
- 15 cell pellet was suspended in 25 ml of 10 mM Tris-Cl pH 8.0, 2mM PMSF, complete protease inhibitor and 15 ug lysozyme. The cells were lysed at 4 °C for 30 minutes, sonicated several times and the lysate centrifuged for 30 minutes at 10,000 x g. The precipitate, which contained the inclusion body, was washed twice with 10 mM Tris-Cl pH 8.0 and 1% CHAPS. The inclusion body was dissolved in 40 ml of 10 mM Tris-Cl
- 20 pH 8.0, 100 mM sodium phosphate and 8 M urea. The solution was bound to 8 ml Ni-NTA (Qiagen) for one hour at room temperature. The mixture was poured into a 25 ml column and washed with 50 ml of 10 mM Tris-Cl pH 6.3, 100 mM sodium phosphate, 0.5% DOC and 8M urea. The bound protein was eluted with 350 mM imidazole, 10 mM Tris-Cl pH 8.0, 100 mM sodium phosphate and 8 M urea. The fractions containing
- 25 FOPP proteins were combined and dialyzed extensively against 10 mM Tris-Cl pH 4.6, aliquoted and stored at - 70 °C.

EXAMPLE 21

REAL-TIME PCR CHARACTERIZATION OF THE PROSTATE-SPECIFIC ANTIGEN P501S IN
PERIPHERAL BLOOD OF PROSTATE CANCER PATIENTS

- 5 Circulating epithelial cells were isolated from fresh blood of normal individuals and metastatic prostate cancer patients, mRNA isolated and cDNA prepared using real-time PCR procedures. Real-time PCR was performed with the TaqmanTM procedure using both gene specific primers and probes to determine the levels of gene expression.
- 10 Epithelial cells were enriched from blood samples using an immunomagnetic bead separation method (Dynal A.S., Oslo, Norway). Isolated cells were lysed and the magnetic beads removed. The lysate was then processed for poly A+ mRNA isolation using magnetic beads coated with Oligo(dT)25. After washing the beads in buffer, bead/poly A+ RNA samples were suspended in 10 mM Tris HCl pH 8.0
- 15 and subjected to reversed transcription. The resulting cDNA was subjected to real-time PCR using gene specific primers. Beta-actin content was also determined and used for normalization. Samples with P501S copies greater than the mean of the normal samples + 3 standard deviations were considered positive. Real time PCR on blood samples was performed using the TaqmanTM procedure but extending to 50 cycles using
- 20 forward and reverse primers and probes specific for P501S. Of the eight samples tested, 6 were positive for P501S and β -actin signal. The remaining 2 samples had no detectable β -actin or P501S. No P501S signal was observed in the four normal blood samples tested.

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EXAMPLE 22

EXPRESSION OF THE PROSTATE-SPECIFIC ANTIGENS P703P AND P501S IN
SCID MOUSE-PASSAGED PROSTATE TUMORS

- When considering the effectiveness of antigens in the treatment of
- 30 prostate cancer, the continued presence of the antigens in tumors during androgen

ablation therapy is important. The presence of the prostate-specific antigens P703P and P501S in prostate tumor samples grown in SCID mice in the presence of testosterone was evaluated as follows.

Two prostate tumors that had metastasized to the bone were removed from patients, implanted into SCID mice and grown in the presence of testosterone. Tumors were evaluated for mRNA expression of P703P, P501S and PSA using quantitative real time PCR with the SYBR green assay method. Expression of P703P and P501S in a prostate tumor was used as a positive control and the absence in normal intestine and normal heart as negative controls. In both cases, the specific mRNA was present in late passage tumors. Since the bone metastases were grown in the presence of testosterone, this implies that the presence of these genes would not be lost during androgen ablation therapy.

EXAMPLE 23

ANTI-P503S MONOCLONAL ANTIBODY INHIBITS TUMOR GROWTH *IN VIVO*

The ability of the anti-P503S monoclonal antibody 20D4 to suppress tumor formation in mice was examined as follows.

Ten SCID mice were injected subcutaneously with HEK293 cells that expressed P503S. Five mice received 150 micrograms of 20D4 intravenously at day 0 (time of tumor cell injection), day 5 and day 9. Tumor size was measured for 50 days. Of the five animals that received no 20D4, three formed detectable tumors after about 2 weeks which continued to enlarge throughout the study. In contrast, none of the five mice that received 20D4 formed tumors. These results demonstrate that the anti-P503S Mab 20D4 displays potent anti-tumor activity *in vivo*.

EXAMPLE 24

CHARACTERIZATION OF A T CELL RECEPTOR CLONE FROM A P501S-SPECIFIC T CELL CLONE

T cells have a limited lifespan. However, cloning of T cell receptor (TCR) chains and subsequent transfer essentially enables infinite propagation of the T

cell specificity. Cloning of tumor-antigen TCR chains allows the transfer of the specificity into T cells isolated from patients that share the TCR MHC-restricting allele. Such T cells could then be expanded and used in adoptive transfer settings to introduce the tumor antigen specificity into patients carrying tumors that express the antigen. T cell receptor alpha and beta chains from a CD8 T cell clone specific for the prostate-specific antigen P501S were isolated and sequenced as follows.

Total mRNA from 2×10^6 cells from CTL clone 4E5 (described above in Example 12) was isolated using Trizol reagent and cDNA was synthesized. To determine Va and Vb sequences in this clone, a panel of Va and Vb subtype-specific primers was synthesized and used in RT-PCR reactions with cDNA generated from each of the clones. The RT-PCR reactions demonstrated that each of the clones expressed a common Vb sequence that corresponded to the Vb7 subfamily. Furthermore, using cDNA generated from the clone, the Va sequence expressed was determined to be Va6. To clone the full TCR alpha and beta chains from clone 4E5, primers were designed that spanned the initiator and terminator-coding TCR nucleotides. The primers were as follows: TCR Valpha-6 5'(sense): GGATCC---GCCGCCACC---ATGTCACCTTTCTAGCCTGCT (SEQ ID NO: 756) BamHI site Kozak TCR alpha sequence TCR alpha 3' (antisense): GTCGAC---TCAGCTGGACCACAGCCGCGAG (SEQ ID NO: 757) SalI site TCR alpha constant sequence TCR Vbeta-7. 5'(sense): GGATCC---GCCGCCACC---ATGGGCTGCAAGGCTGCTCT (SEQ ID NO: 758) BamHI site Kozak TCR alpha sequence TCR beta 3' (antisense): GTCGAC---TCAGAAATCCTTTCTCTTGAC (SEQ ID NO: 759) SalI site TCR beta constant sequence. Standard 35 cycle RT-PCR reactions were established using cDNA synthesized from the CTL clone and the above primers, employing the proofreading thermostable polymerase PWO (Roche, Nutley, NJ).

The resultant specific bands (approx. 850 bp for alpha and approx. 950 for beta) were ligated into the PCR blunt vector (Invitrogen) and transformed into *E. coli*. *E. coli* transformed with plasmids containing full-length alpha and beta chains were identified, and large scale preparations of the corresponding plasmids were generated. Plasmids containing full-length TCR alpha and beta chains were submitted

for sequencing. The sequencing reactions demonstrated the cloning of full-length TCR alpha and beta chains with the determined cDNA sequences for the Vb and Va chains being shown in SEQ ID NO: 760 and 761, respectively. The corresponding amino acid sequences are shown in SEQ ID NO: 762 and 763, respectively. The Va sequence was
5 shown by nucleotide sequence alignment to be 99% identical (347/348) to Va6.2, and the Vb to be 99% identical to Vb7 (336/338).

From the foregoing it will be appreciated that, although specific
embodiments of the invention have been described herein for purposes of illustration,
10 various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is Claimed:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 and 786-788;

(b) complements of the sequences provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 and 786-788;

(c) sequences consisting of at least 20 contiguous residues of a sequence provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 and 786-788;

(d) sequences that hybridize to a sequence provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 and 786-788 under moderately stringent conditions;

(e) sequences having at least 75% identity to a sequence of SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-

375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 and 786-788;

(f) sequences having at least 90% identity to a sequence of SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 and 786-788; and

(g) degenerate variants of a sequence provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 and 786-788.

2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 112-114, 172, 176, 178, 327, 329, 331, 336, 339, 376-380, 383, 477-483, 496, 504, 505, 519, 520, 522, 525, 527, 532, 534, 537-551, 553-568, 573-586, 588-590, 592, 627-629, 632, 633, 635, 637, 638, 656-671, 675, 683, 684, 710, 712, 714, 715, 717-719, 723-734, 736, 740-750, 752, 754, 755, 766-772, 777-785 and 789-791;

(b) sequences having at least 70% identity to a sequence of SEQ ID NO: 112-114, 172, 176, 178, 327, 329, 331, 336, 339, 376-380, 383, 477-483, 496, 504, 505, 519, 520, 522, 525, 527, 532, 534, 537-551, 553-568, 573-586, 588-590, 592, 627-629, 632, 633, 635, 637, 638, 656-671, 675, 683, 684, 710, 712, 714, 715, 717-719, 723-734, 736, 740-750, 752, 754, 755, 766-772, 777-785 and 789-791;

(c) sequences having at least 90% identity to a sequence of SEQ ID NO: 112-114, 172, 176, 178, 327, 329, 331, 336, 339, 376-380, 383, 477-483, 496, 504, 505, 519, 520, 522, 525, 527, 532, 534, 537-551, 553-568, 573-586, 588-590, 592, 627-

629, 632, 633, 635, 637, 638, 656-671, 675, 683, 684, 710, 712, 714, 715, 717-719, 723-734, 736, 740-750, 752, 754, 755, 766-772, 777-785 and 789-791;

- (d) sequences encoded by a polynucleotide of claim 1;
- (e) sequences having at least 70% identity to a sequence encoded by a polynucleotide of claim 1; and
- (f) sequences having at least 90% identity to a sequence encoded by a polynucleotide of claim 1.

3. An expression vector comprising a polynucleotide of claim 1 operably linked to an expression control sequence.

4. A host cell transformed or transfected with an expression vector according to claim 3.

5. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a polypeptide of claim 2.

6. A method for detecting the presence of a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with a binding agent that binds to a polypeptide of claim 2;
- (c) detecting in the sample an amount of polypeptide that binds to the binding agent; and
- (d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient.

7. A fusion protein comprising at least one polypeptide according to claim 2.

8. The fusion protein of claim 7, wherein the fusion protein comprises a sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO: 682, 692, 695, 699, 703 and 709; and

(b) sequences encoded by SEQ ID NO: 679, 691, 696, 700, 704 and 708.

9. An oligonucleotide that hybridizes to a sequence recited in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-626, 630, 631, 634, 636, 639-655, 674, 680, 681, 711, 713, 716, 720-722, 735, 737-739, 751, 753, 764, 765, 773-776 or 786-788 under moderately stringent conditions.

10. A method for stimulating and/or expanding T cells specific for a tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

(a) polypeptides according to claim 2;
(b) polynucleotides according to claim 1; and
(c) antigen-presenting cells that express a polypeptide according to claim 1,

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

11. An isolated T cell population, comprising T cells prepared according to the method of claim 10.

12. A composition comprising a first component selected from the group consisting of physiologically acceptable carriers and immunostimulants, and a second component selected from the group consisting of:

- (a) polypeptides according to claim 2;
- (b) polynucleotides according to claim 1;
- (c) antibodies according to claim 5;
- (d) fusion proteins according to claim 7;
- (e) T cell populations according to claim 11; and
- (f) antigen presenting cells that express a polypeptide according to claim 2.

13. A method for stimulating an immune response in a patient, comprising administering to the patient a composition of claim 12.

14. A method for the treatment of a cancer in a patient, comprising administering to the patient a composition of claim 12.

15. A method for determining the presence of a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with an oligonucleotide according to claim 9;
- (c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and
- (d) compare the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient.

16. A diagnostic kit comprising at least one oligonucleotide according to claim 9.

17. A diagnostic kit comprising at least one antibody according to claim 5 and a detection reagent, wherein the detection reagent comprises a reporter group.

18. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4+ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of: (i) polypeptides according to claim 2; (ii) polynucleotides according to claim 1; and (iii) antigen presenting cells that express a polypeptide of claim 2, such that T cell proliferate; and

(b) administering to the patient an effective amount of the proliferated T cells,

thereby inhibiting the development of a cancer in the patient.

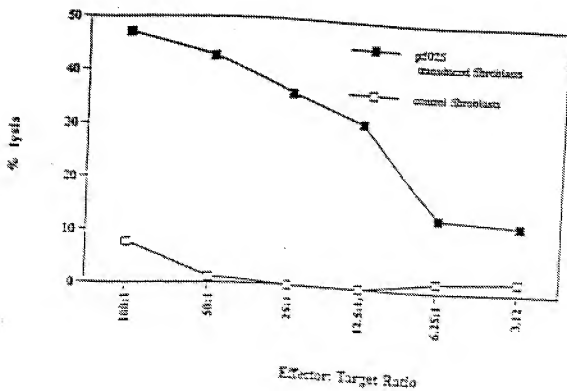


Fig. 1

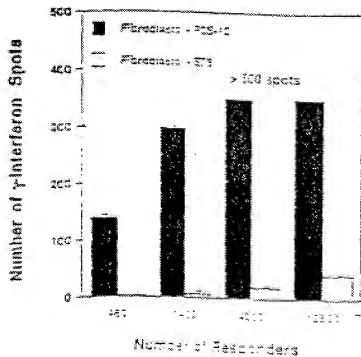


FIG. 2A

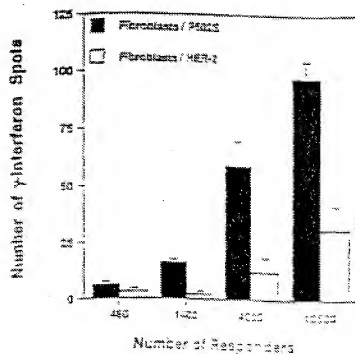


Fig. 25

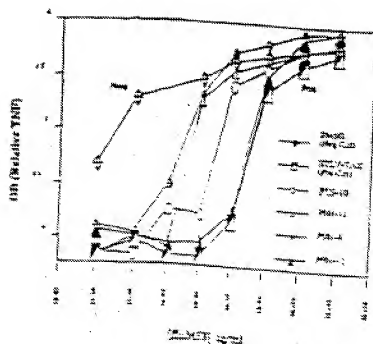


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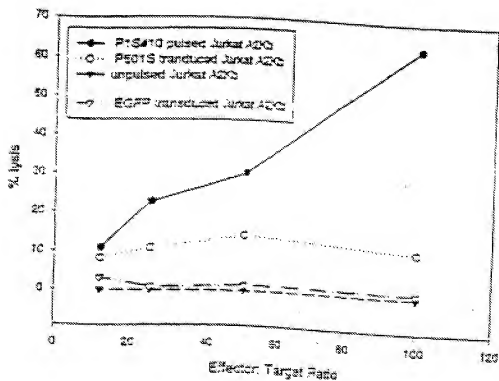


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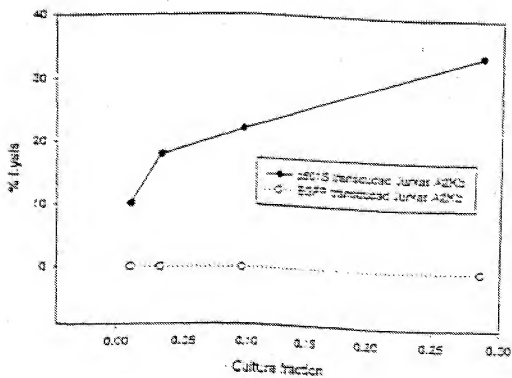


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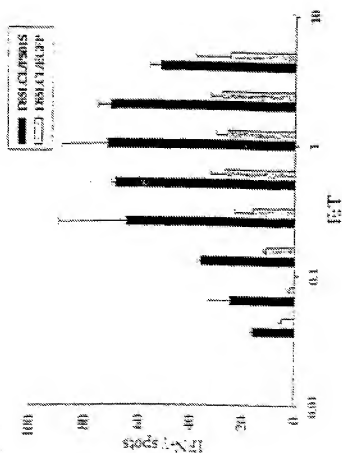


Fig. 6B

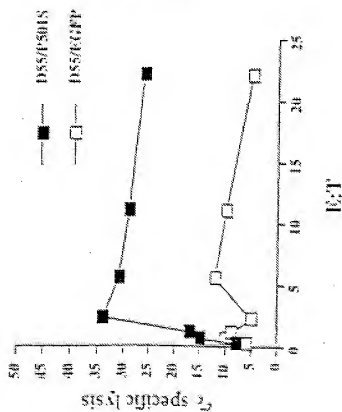
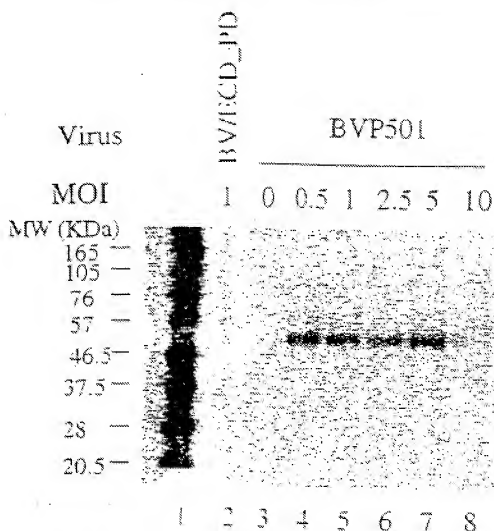


Fig. 6A

Expression of P501S by the Baculovirus Expression System



0.6 million 293T cells in 6-well plate were infected with an unrelated control virus BV/ECD_PD (lane 2), without virus (lane 3), or with recombinant baculovirus for P501 at different MOI (lane 4-8). Cell lysates were run on SDS-PAGE under the reducing condition and analyzed by Western blot with a monoclonal antibody against P501S (P501S-10E3-4D3). Lane 1 is the biotinylated protein molecular weight marker (BioLabs).

Fig. 7

Figure 1. Schematic of P501S with predicted transmembrane, cytoplasmic, and extracellular regions

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Unfolded sequence: Predicted transmembrane domain; Bold sequence: Predicted extracellular domain;

Biotic sequence: Predicted intracellular domain. Sequence in bold/underlined: used to generate polyclonal rabbit serum

Localization of domains predicted using IMMTOPI (G.P. Tlstady and I. Simon (1998) Principles

of Growing Antibody Acid Composition of Integral Membrane Proteins: Applications to immunology Prediction. J Mol Biol. 283, 489-506.

Genomic Map of (5) Corixa Candidate Genes

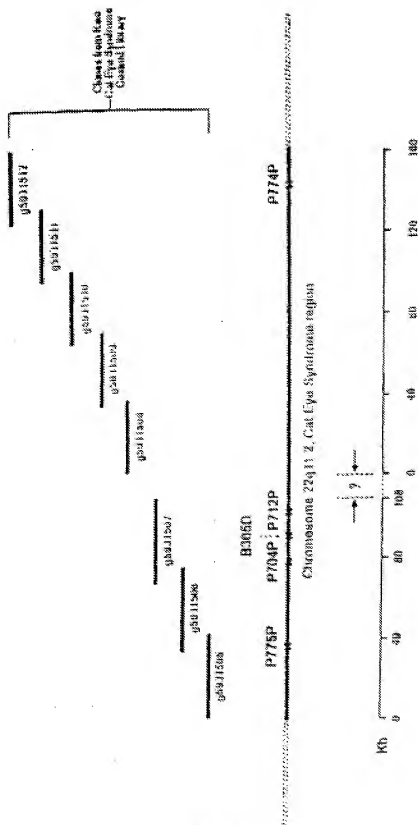


FIGURE 4. Elisa assay of rabbit polyclonal antibody specificity

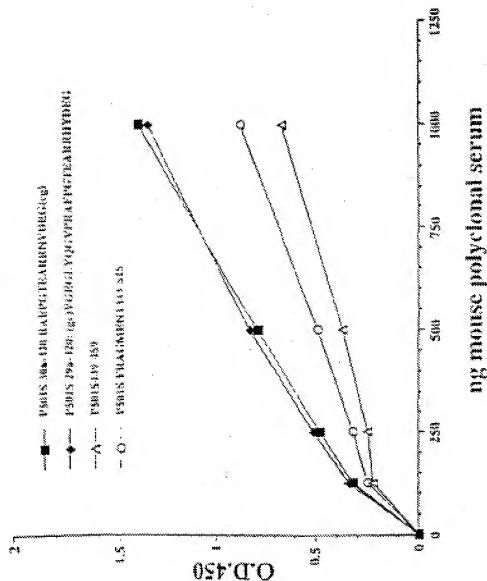


Fig. 11

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 Kalos, Michael D.
 Fanger, Gary R.
 Retter, Marc W.
 Stolk, John A.
 Day, Craig E.
 Skeiky, Yasir A.W.
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| gtctacntcc | ccatnaccnc | gnctcnatgg | ancntccnt | tttaannacc | tctnnaactt | 660 |
| gggaananc | ctcgncnta | ccccctttaa | tcncccttg | cnangnncnt | ccccnntcc | 720 |
| ncnccntng | cnctntnann | caaaaaagcc | cnncnccaa | tctcctnccn | ccctccttcc | 780 |
| ccnccctccg | aaatcgcccn | c | | | | 801 |

<210> 10
 <211> 789
 <212> DNA
 <213> Homo sapien
 <220>
 <221> misc_feature
 <222> (1)...(789)
 <223> n = A,T,C or G

| | | | | | | |
|-------------|------------|------------|-------------|------------|------------|-----|
| cagctctatnt | ggccagtggt | gcagctttcc | ctgtgggtgc | gggtggccca | tgcctgtccc | 60 |
| acagtgctggc | ogtggtgaca | gcttcagccc | ccctcaacgg | gttcaccttc | tcagccctgc | 120 |
| agatccctgc | ctacacactg | gctccctct | accaacggcc | gaagcaggtg | tctctgccca | 180 |
| aatccagagg | ggacactgga | ggtgctagca | gtgaggaacg | ctgtatgacc | agcttctctg | 240 |
| caggccctaa | gcttgagct | cccttcccta | atggacaagt | gggtgtctga | ggcagtggcc | 300 |
| tgtctccacc | tcacnccgg | ctctgggggg | ccctgtccctg | tgatgtctcc | gtacgtgtgg | 360 |
| tgttgggtga | gcccaaccan | gccaggggtg | ttccggggcg | gggcctctgc | ctggacactg | 420 |
| ccatccctga | tagtgtcttc | tgtgtctcca | ngtggcccca | tcctgtttta | tgggtcccat | 480 |
| tgtccagctc | agccagctcg | tcactgccta | tatgtgtct | gcgcagggcc | tgggtctgtg | 540 |
| cccttttact | tgtctacac | ggtantattt | gacaagaacg | anttggccaa | atactcagcg | 600 |

| | | | | | | |
|------------|------------|------------|------------|------------|------------|-----|
| ttaaaaaatt | ccagcaacat | tgggggtgga | aggcctgcct | cactgggtgc | aactccccgc | 660 |
| tccgtttaac | cccaggggg | tgcgggctg | gccgcgaatt | tctgttgctg | ccaaantnat | 720 |
| gtggctctct | gtgcccacct | gttgctgctg | gaagtgcnta | cnccnccant | nggggggtng | 780 |
| ggngttccc | | | | | | 789 |

<210> 11
 <211> 772
 <212> DNA
 <213> Homo sapien

 <220>
 <221> misc_feature
 <222> (1)...(772)
 <223> n = A,T,C or G

| | | | | | | |
|-------------|------------|------------|------------|------------|------------|-----|
| <400> 11 | | | | | | |
| cccaccctac | ccaaatatta | gacaccaaca | cagaaaagct | agcaatggat | tcctctctac | 60 |
| tttgttaaat | aaataagtta | aatattttaa | tgcctgtgtc | tctgtgatgg | cacacagaag | 120 |
| accaacaggc | ccatctctga | taaaaggtaa | gaggggggtg | gatcagcaaa | aagacagtgc | 180 |
| tgtgggctga | ggggccctgg | tcttctgtgt | tggccctcca | ggactctctc | cctacaataa | 240 |
| actttcatat | gttcaaatcc | catggaggag | tgtttcatcc | tagaacctcc | catgcaagag | 300 |
| ctacattaaa | cgaagctgca | ggttaagggg | cttanagatg | ggaaaaccag | tgaactgagt | 360 |
| tattcgctgc | ccaaaaacc | tctcttaggt | gtgtctcaac | taggaggcta | gctgttaacc | 420 |
| ctgagcctgg | gtaatccacc | tgcagagctc | cgcattccca | gtgcatggaa | ccctctctgg | 480 |
| ctccctgtat | aagtcacagc | tgaiaacccc | ttggaaggnc | tccagtcagg | cagccctana | 540 |
| aactggggga | aaaagaaaa | gagcccccac | cccacagctg | tgcunctaag | caactcaaca | 600 |
| gcacaggggtg | gcagcaaaaa | aaccacttta | ctttggcaca | aaacaaaact | ngggggggca | 660 |
| accccggcac | cccnangggg | gttaacagga | ancngggnaa | cntggaaacc | aattnaggca | 720 |
| ggcccccac | cccaatnttt | gctgggaast | ttttccctcc | ctaatnttt | tc | 772 |

<210> 12
 <211> 751
 <212> DNA
 <213> Homo sapien

 <220>
 <221> misc_feature
 <222> (1)...(751)
 <223> n = A,T,C or G

| | | | | | | |
|------------|-------------|------------|-------------|-------------|-------------|-----|
| <400> 12 | | | | | | |
| gcccacattc | cagctgccac | accaccccag | gtgactgcct | tagttgggat | gtcatadcaa | 60 |
| agctgattga | agcaacccct | tacttttttg | tctgtgagcct | tttgccttgg | gcaggttttc | 120 |
| tgtgctgtgt | tggtgacgtt | gtcattgcac | cagaatgggg | gaaaggaact | gtctcttttg | 180 |
| aagtanggtg | agctctccaa | atccgtatat | tgtgtgaagc | cacagcactt | gagccctttc | 240 |
| attgttggtg | tccacacttg | agtgaagtct | tccctgggaa | ctaatctttt | cttgatggca | 300 |
| ggcactacca | gcaacgtcag | ggaagtgtct | agccattgtg | gtgtacacca | agggagccac | 360 |
| agcagctgcn | accctcagca | tgaagatgan | gaggangatg | aagaagaacg | tccagagggc | 420 |
| acacttgcct | tcagttcttan | ccccatncca | gcoontgaaa | accaanacca | aagaccacna | 480 |
| cnccggctgc | gatgaagaaa | tnacccccng | ttgacaaact | tgcattggca | tgggancacc | 540 |
| agtggtccna | aaaactttca | aaaaggatgc | cccatcnatt | gaccccccaa | atgcccaactg | 600 |
| ccaacagggg | gtcccccccn | cnccnaacga | tgancncatt | gnaccaagctc | tcnctggctct | 660 |
| tnactnaact | gaacccctgc | tngtggctcc | gtttcaggnc | cnnggocctg | cttctnaann | 720 |
| aangaactcn | gaagncccc | cnnganann | g | | | 751 |

<210> 13
 <211> 729
 <212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(729)

<223> n = A,T,C or G

<400> 13

| | | | | | | |
|-------------|------------|-------------|-------------|-------------|------------|-----|
| gagcagggcg | tccctctgoc | tgcccaactca | gtggcaaacac | cogggagctg | ttttgtcctt | 60 |
| tgtyganctc | cagcagtnoc | ctctttcaga | actcantgoc | aaganccctg | aacaggagcc | 120 |
| accatgcagt | gcttcagctt | cattaagaac | atgatgatcc | tcttcaattt | gctcctcttt | 180 |
| ctgtgtgggt | cagccctgtt | ggcagtgagg | atctgggtgt | caatcgatgg | ggcctacctt | 240 |
| ctgaagatct | tggggccact | gtctgtccagt | gcctatgcagt | ttgtcaacgt | gggctaactc | 300 |
| ctcatcgacg | cogggcgttt | ggtcttagct | ctaggtttcc | tgggctgcta | tggtgctaag | 360 |
| actgagagca | agtgtgcctc | cgtgacgttc | ttcttcatcc | tctctcctat | cttcatgtgt | 420 |
| gaggttgcaa | tgctgtgggt | gcttgggtgt | acaccacaat | ggctgagcac | ttcctgacgt | 480 |
| tgctggtaat | gcctgccatc | aanaaasgat | tatgggttcc | caggaanact | tcactcaagt | 540 |
| gttggaaacac | caccatgaaa | gggctcaagt | gctgtggctt | cnnccaaacta | tacggatttt | 600 |
| gaagantcac | ctacttcaaa | gaaanagtg | cttttccccc | attctgtgtg | caattgacaa | 660 |
| acgtccccaa | caacgccaat | tgaaaacctg | caccacaacc | saanggggtc | ccaccanana | 720 |
| attnaaggg | | | | | | 729 |

<210> 14

<211> 816

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(816)

<223> n = A,T,C or G

<400> 14

| | | | | | | |
|-------------|------------|------------|------------|-------------|-------------|-----|
| tgctcttctc | caaaagtgtt | cttgttgcaa | taacaaccac | catagggtaaa | gogggcgccg | 60 |
| tgttcgctga | aggggttgta | gtaccagcgc | gggatgtctc | ccttgccagc | tcctgtgtct | 120 |
| ggcaggtcca | cgcagtgcoc | tttgtcactg | gggaaatgga | tggcctggag | ctcgtcaaat | 180 |
| ccactcgtgt | atthttcaac | ggcagcctcg | tcgcagcgtg | cggggcagtt | gggggtgtct | 240 |
| tcacactcca | ggaaactgtc | natgcagcag | ccattgtctc | agcggaactg | gggtggcctg | 300 |
| cangtgcacg | agccactcgt | atgggcctct | tcctatgnan | gggcctctgng | ggaaagtccc | 360 |
| tganeccaan | ancctgcctc | caaanccccc | accttgcaaa | cccgcacagg | ctagaatgga | 420 |
| atcttctctc | cgaaaggtag | ttntttctgt | tgcccaancc | anccccntaa | acaaactctt | 480 |
| gcanaatctc | tcggnggggg | tcntantacc | anctgtggaa | aagaacccca | ggcngcgaaac | 540 |
| ccancttctt | tggatnccga | gcnaataatc | ncntttctgc | ttgtgtggaca | gcaccantna | 600 |
| ctgttnanct | ttagneantc | gtctctntgg | gttgnncttg | aacctaaten | ccnntcaact | 660 |
| ggggacaagg | aantngcctt | ccttttaatt | cccnanctna | ccccctgggt | tgggggtttt | 720 |
| cncctctcta | cccccgaaaa | acgtgttccc | cccccaacta | ggggccnasa | ccnnttnttc | 780 |
| ccacacccctn | ccccccccc | gggtctngnt | ggttng | | | 816 |

<210> 15

<211> 783

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(783)

<223> n = A,T,C or G

<400> 15

| | | | | | | |
|------------|------------|------------|------------|------------|-------------|-----|
| ccaaggccct | ggcaggcata | nacttgaagg | tacaacccca | ggaacccctg | gtgctgaagg | 60 |
| atgtgaaaa | cacagattgg | cgctactgc | gggtgacac | ggatgtcagg | gtagagagga | 120 |
| aagaccaca | ccagggtgaa | ctgtggggac | toaaggaang | cacctacctg | ttccagctga | 180 |
| cagtgaactg | ctcagaccac | ccagaggaca | cggaacaagt | cacagtccct | gtgctgtcca | 240 |
| ccaagcgagc | agaagactac | tgcctcgcat | ccaacaangt | gggtogctcg | cggggctott | 300 |
| tcaccgctg | gtactatgac | ccaacggagc | agatctgcaa | gagttkgtt | tatggagggt | 360 |
| gcttgggcaa | caagaacaaa | taccttcggg | aagaagagtg | cattctancc | tgtcnggggtg | 420 |
| tgcaagtggt | goccttgana | ngcanctctg | gggtcangc | gaettccccc | caaggccctc | 480 |
| ccatgggaag | ggcgcataca | atgttctctg | gcacctgtca | gcccacccag | ttccgctgca | 540 |
| ncaatggctg | ctgcatcnac | antttccctg | aatttggaca | accccccccc | ntgccccaaa | 600 |
| ccctcccaac | aaagcttccc | tgtnaaaaaa | tacnccantt | ggcttttnac | aaacnccggg | 660 |
| cnctccntt | ttcccnntn | aacaaagggc | ncnngenttt | gaactgccc | aaacnaggaa | 720 |
| tctnccnegg | aaaaantncc | ccccctggtt | octnnaanco | octccncaa | anctnccccc | 780 |
| ccc | | | | | | 837 |

<210> 16

<211> 801

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(801)

<223> n = A,T,C or G

<400> 16

| | | | | | | |
|-------------|------------|------------|------------|-------------|-------------|-----|
| gccccaatc | cagctgcac | accacccagc | gtgactgcat | tagttcggat | gtcatccaaa | 60 |
| agctgattga | agcaacccct | tactttttgg | tctgagccct | tttgcttggg | gcagggttca | 120 |
| ttgggtgtgt | gtctgacgtt | gtcattgcaa | cagaatgggg | gaaaggccact | gtttctctttg | 180 |
| aagtaggggtg | agctcctaaa | atccgtatag | ttggtgaaac | ccagaccatt | gagccctttc | 240 |
| atggttggtg | tcacaccttg | agtgaagtct | tactgggaac | cataatcttt | cttgatggca | 300 |
| ggcactccca | gcaacgtcag | gaagtgtctc | gccttcttgg | tgtacaccaa | ggcgaccaca | 360 |
| gcagctgcaa | cctcagcaat | gaagatgagg | aggaggtatg | agaagaaact | cncgagggca | 420 |
| cacttgcctc | cgtctctagc | accatagcag | cccangaaac | caagagccaa | gaccccaacg | 480 |
| ccagctcgca | atgaagaaga | ntacccacgt | tgacaaaact | catggccact | ggaagacagt | 540 |
| tgcccccgaan | atcttcagaa | aagggtgccc | ccatcgattg | aacaccana | tgcccaactgc | 600 |
| cnacagggtgt | gcnccnccn | gaaagaatga | gccattggaq | aaggatcttc | ntggtcttaa | 660 |
| tgaactgaaa | ctctgatgg | tggccctctg | tcagggtctc | tggcagtgaa | ttctgaaaaa | 720 |
| aaggacnccg | atnagccccc | ccaaagana | aaacacccc | gggtgttgcc | ctgaattggc | 780 |
| ggccagggan | ccctgcccnc | g | | | | 801 |

<210> 17

<211> 740

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(740)

<223> n = A,T,C or G

<400> 17

| | | | | | | |
|------------|------------|------------|------------|------------|------------|-----|
| gtgagagcca | ggcgtccctc | tgcctgcaca | ctcagtgcca | acacccggga | gctgttttgt | 60 |
| cccttttgga | gcctcagcag | ttccctcttt | cagaactcac | tgcaacagcg | octgaacagc | 120 |
| agccacactg | cagtgcttca | gcttcattaa | accatgatg | atcctcttca | atttgtcatc | 180 |
| ctttctgtgt | ggtgcagccc | tgttggcagt | gggaatctgg | gtgtcaatcg | atggggcacc | 240 |
| ctttctgaag | atctctgggc | cactgtcgtc | cagtgccatg | cagtttgta | acgtgggcta | 300 |

| | | | | | | |
|------------|------------|-------------|------------|------------|------------|-----|
| cttcctcctc | gcagccggcg | ttgttggtctt | tgctcttggg | ttcttggggt | gctatgggtc | 360 |
| taagacggag | agcaagtgtg | cctctgtgac | gttctctctc | atcctctctc | tctctctctc | 420 |
| tgctgaagt | gcagctgctg | tqgtcgccct | ggtgtacacc | acaatggctg | sacctctctc | 480 |
| gcagctgctg | gtantgctgt | ccatcaaaaa | agattatggg | ttcccgagga | saattcactc | 540 |
| aanlttgga | caacnccatg | aaaagggtct | caattttctg | tggtttcccc | aactataccg | 600 |
| gaattttgaa | agantcnccc | tacttccaaa | aaaaaanant | tgctttttcc | ccctttctgt | 660 |
| tgcaatgaaa | acntcccaan | acngcccaan | aaaacctgcc | cnnncaaaaa | ggntcncaaa | 720 |
| caaaaaaant | naaagggttn | | | | | 740 |

<210> 18

<211> 802

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(802)

<223> n = A,T,C or G

<400> 18

| | | | | | | |
|------------|-------------|------------|------------|------------|------------|-----|
| ccgctggttg | cgctgggtaca | gngnagccac | gaagcacgtc | agcctacaca | gcctcaatca | 60 |
| caaggtcttc | cgagctgcgc | acattacgca | gggcaagagc | ctccagcaac | actgcatacg | 120 |
| ggatacactt | tactttagca | gccagggtga | caactgagag | gtgtogaagc | ttattctctc | 180 |
| gagcctctgt | tagtggagga | agattccggg | cttcagctaa | gtagtccagc | tatgtcccat | 240 |
| aagcaaaacc | tgtgagcagc | cgggaagtag | aggcaaaatc | actctcagcc | agctctctaa | 300 |
| cattgggcac | gtccagcagc | tctccaaaac | cgtagavacc | agnngcctcc | agcacctgat | 360 |
| ggatgagtgt | ggccagcgct | gcccctcttg | cgcacttggt | taggagcaga | aattgtctct | 420 |
| ggttctgccc | tgctaccttc | acttccgcac | tcatacaetg | actgagtgtg | ggggactttg | 480 |
| gtccaggtat | tcocagagag | tggttccgcc | ccctccttta | atgacacnng | ccanncaacc | 540 |
| gtcgctctcc | gcccantgng | ttcgtctgnc | ctgggtccag | gtctgtctgg | cnctaacttg | 600 |
| aancttctgt | nggcccattg | aattcacncc | accggaaact | gtangabcca | ctantctctc | 660 |
| aaccggncgc | caccgcnntt | ggaactccac | tcttnttacc | tttactgtag | gggttaagtc | 720 |
| accccttncc | ttaccttggc | ccaaacnctn | ccatgtgtcg | anattngtaa | tcngnccnca | 780 |
| tnccanccnc | atangaagcc | ng | | | | 802 |

<210> 19

<211> 731

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(731)

<223> n = A,T,C or G

<400> 19

| | | | | | | |
|------------|-------------|------------|------------|------------|------------|-----|
| cnaagcttcc | aggttaccggg | cgcnaaaccc | tgaccnagg | tancanaang | cagncngcgg | 60 |
| gagcccccgc | tcacngngmg | ngtctcttat | nggagggggc | ggagccacat | cnctggacnt | 120 |
| cntagcccca | actcccccnc | ncncantgca | gtgatggagt | cagaactgaa | ggttncgttg | 180 |
| caggaaacca | gancaaaanc | tgctccnntc | caagtccggc | nagggggcgg | ggctggccac | 240 |
| gncactccat | cnagtgtcgn | aaagccccc | cctgtctact | tggttgagga | cncgcnnga | 300 |
| cattgcccag | gttanataac | nggcnagag | tnantttgoc | tctcccttcc | ggctgocgan | 360 |
| cngtintgtg | tagnngacat | aacctgacta | cttaactgaa | ccnngaatc | taconccact | 420 |
| ccactaaagt | cagaaacaaa | aacttcgaca | ccactcact | gtcacctgnc | tgctcaagta | 480 |
| aagtgtaccc | caatnccaat | gtnctgtaga | ngctctgnc | tgcacttngt | tcggtccgtg | 540 |
| gaagacotat | caattnaagc | tatgtttctg | actgctcttt | gctccctgna | acaanonaac | 600 |
| cnncnftcca | agggggggnc | ggcccccaat | cccccaacc | ntnaattnan | tttancoccc | 660 |
| ccccngggcc | cggcctttta | cnancntcnn | nnaengggna | aaacccnngc | tttncocaa | 720 |

nnastocnoo t

731

<210> 20
 <211> 754
 <212> DNA
 <213> Homo sapien
 <220>
 <221> misc_feature
 <222> (1)...(754)
 <223> n = A, T, C or G

<400> 20
 tttttttttt tttttttttt taataaacccc ctccattnaa tgnaaacttc agaaattgtc 60
 caaccccctc ntccaaatnn ccttttccgg gngggggttc caaacccaan ttanntttgg 120
 annttaaat aaatntntnt tggnggnnaa anccnaatgt nangaasgtt naaccanta 180
 taacttnnaa tncctggaaa cngtngntt ccaaaaatnt ttaaccotta antccctccg 240
 aaatngttna nggaaaaccc aantctctnt aaggttgttt gaaggnntnaa tnaaaanccc 300
 nnccaattgt ttttngccac goctgaatta atggnttcc gntgttttcc nttaaaanaa 360
 ggnnaacccc ggttatannaa tccccccnnc ccaattata coganntttt ttingaattgg 420
 gancocnccg gaattaacgg gganntccc tnttgggggg cnggnncccc ccccnteggg 480
 ggttnggggc aggnnnaaat tgtttaaggg tccgaaaaat cctccnaga aaaaaanctc 540
 ccaggttgag nntnggtgtt nccccccccc canggccctc ctcgmanagt tgggggtttgg 600
 ggggcoctggg stttttnttc cctnttnc tccccccccc cngggganag aggttngnt 660
 ttgtctnnc ggcccccncn aaganccttn coganntnaa ttaaatccnt goctnggcga 720
 agtctnttg agggntaaan ggcocccctnn cggg 754

<210> 21
 <211> 755
 <212> DNA
 <213> Homo sapien
 <220>
 <221> misc_feature
 <222> (1)...(755)
 <223> n = A, T, C or G

<400> 21
 atcancocat gaccocnasc nngggacncc tcanccggnc hnnncnccnc cggccnatca 60
 nngtnagnnc actncaatna naccacnccc cncnactac gcccnncnnc cnaagcncta 120
 nncanatincc actganngcg cganngtngan ngagaaanct nataccanag ncaaccanacn 180
 ccaegtgtcc nanaangcct nnnatacngg nnnatccaat ntgnanccct cnaagtattn 240
 nncmncanct gatttccctn anocgattac cctncccccc tancccccct cccccaaana 300
 cgaagcncct ggnccnaagc nhgcgncccc ccctagntc cccncaagt cncncncta 360
 aactcaoncn nattaonccg ttontgagta tcactcccc aatctcccc tactcaacte 420
 aaaaaatcn gatataaat aatcacagcc tgaattatnc actntgactg gytctctatt 480
 ttagnngtcc ntnaancncc ctaataacttc cagtctncc tcncccaatt cnaaangcct 540
 ctttngaca gcatnttttg gttcccnatt gggttctlan agaatggccc ttontngaac 600
 gggctcctct tttctctgg ttanccctgg tcncccgcc cagtattat ttcctnttt 660
 aattctnnc cttttanttt tggontttna aaccccggcc cttgaaaaag gccccctgg 720
 aaaaaggtgt tttganaaaa tttttgtttt gtccc 755

<210> 22
 <211> 849
 <212> DNA
 <213> Homo sapien

<220>

```

<221> misc_feature
<222> (1)...(849)
<223> n = A,T,C or G

<400> 22
tttttttttt ttttttangtg tngtctgtgca ggtagagggt tactaceant gtgaanacgt      60
aogctnggan taangcgaac cgaattctag gannmccct aaaatcanac tgtgaagatn      120
atcctganna cgggaanggt accggngat ntgtctaggg tgnmccctcc cannncntta      180
cataacteng nggcoctgoc caccaccttc ggcgggccng ngncogggcc ogggtcattn      240
gnattaacon cactnngcna nogggttcon ncccnncag aeccgggcga tccgggggtnc      300
tgtgtcttcc cctnngncon aaaaantggg ccnccggccc cttaacccct nnacaagcca      360
engcctcta ncnncngccc cccctccaut nngggggact gcmanngct ccgttcttag      420
nnaccccnnn gggttactag gttgtcgant cnaccgnang ccanggatc cnaaggaggg      480
tgogttnttg gccctaccc ttccctnogg nncacccctc ccgaananga nccgctcccg      540
cnccnngang cctcnccctg caacacccgc nctcctcgt nccgnnnccc ccccaacccg      600
nccctcnccn ngngnangcn ctccnccncc gtctcanna caaccccgcc ccgcaaggcc      660
atcncnccan ggngnangcn ngcnccntc gccccggcgn gcgnccctc cgcncnngaa      720
ctactcngg ccantnccg tcacnccnna cnaaacgcgc ctgcggcgcc cgaagcgccc      780
acctcncga gtccctccgg ctccnacc angnctcon ccaggscacn nnaccccgcc      840
nnccagcgg

<210> 23
<211> 872
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(872)
<223> n = A,T,C or G

<400> 23
ggcgaacta tactctgctg gnaactcgtc gctctcgtnc tottttcttc cgcacacatg      60
tgtgacnanc cagattnggc ngatatcnan aagntcgenc agtccaaact gantaacaca      120
cacacnccan aganaaatcc nctgccttcc anagtanacn attgaacnng agaaccangc      180
ggcggaatcg taatnaggcg tgcgcgcaca atntgtcncc gttlatntn ccagcntcnc      240
ctacnacc ccactctctn nagctgtcnn acccctngtn cgnacccccc naggtcggga      300
tcgggttttn nntgacccng cnnccctccc cccctccat naoganccac ccgcaccacc      360
aaangcnccg accccgcmct ctccgcacnc ctgtcctata cccctgtngc ctggcnmngn      420
acogcaatga cctctgcenn ctncnngaaa ncgnanacgt ccgggttgna amnanogctg      480
tgggnangcg tctgcncocg gttccttcon ncncttccca ccatctctnt taacngggtct      540
ccnccgcnct tcnnnacncc cctgggaagc tntcctntgc cccctttnac tccccccctt      600
cgnogtgncc cgcnccccac ntcattncca naagntcttc acaannnccct ggatnnctcc      660
cncnccnccn gtcancnccg ggaagggngg ggncccnntg nttagcgttg ngngnangtc      720
cgaanantcc tcnccntcan cactacccct cggggcgnct ctongttncc accttancaa      780
ntctcccccg ngngcnctcc tccagctcnc cccccccct ctctgcantg tncctctgct      840
tnacnntac gantnttccn cncctcttt cc

<210> 24
<211> 815
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(815)
<223> n = A,T,C or G

```



```

<400> 24
gcatgcaagc ttgagtattc tatagnngta cctaaatanc ttggcntaat catggctnta      60
nctgncttcc tgtgtcaaat gtatacnaaa tanstatgaa tctnatntga caagannnta      120
tcntncatta gtaacaaant tnttgtccat cctgtcngaa caaattccca tnnattncgn      180
cgcattcnnc gncantatn taatngggaa ntcnntnnn naacnnacat ctatcnncc      240
gncocctgac tgganagatg ggaatnattc tntntgacc naatgttcca tcttggtatn      300
aanancoccc cgcngnccac cgggtngnng cnagcennnc caagacccca ctgtggaggt      360
aaactgcgtc aganncatca aacntgggaa acccgcnncg angtnnaagt ngnnncnaan      420
gatccgtccr agnttnaoc ctcccttenc agcgccccc ttingtgcctt anagnnagc      480
gtgtcnanac nctcaacac ganacgcgcc agnccanccg caattnggca caatgtcgnr      540
gaaccccca ggggganina tncanaccc caggattgtc cncncangaa atcccnanc      600
ccnccctac ccnactttg gacngtgacc aantcccgga gtncacgtcc ggcngnctc      660
cccccacggt nncntgggg ggtgaaact cngntcanc cngnccaggn ntcgnaagga      720
accggccctn ggcgaanng ancnntenga agncccnct cgtataaccc cccctcncca      780
nccnaengnt agntccccc cngggtacgg aangg      815

```

<210> 25

<211> 775

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> {1}...{775}

<223> n = A,T,C or G

<400> 25

```

ccgagatgtc tgcgtccgtg gccttagctg tgcctgcgct acctctctctt tctggcctgg      60
aggtatccca cggtaactcca aagattcagg tttaactcac tcatccagca ggaatggaa      120
agtcgaattt cctgaattgc tatgtgtctg ggtttcatcc atccgcatt gaanttgact      180
tactgaagaa tgganagaga attgaataag tggagcattc agactgtctc ttacgcaagg      240
actggtcttt ctatctctng tactacactg aattcaaccc cactgaaaaa gatgagtatg      300
cctgcgctgt gaacaaatgt actttgtcac agcccaagat agttaagtgg gatcgagaca      360
tgtgaacagn cmncatggaa gtttgaagat gcgcacattg gattgggtga attccaaatt      420
ctgcttgctt gctttttaa antgatstgc ntataacccc taacctttat gcccaccaatt      480
tgtagggggt acatnangt tcnctnngga catgatcttc cttkataant cncncttgc      540
aattgcocgt cncocngtna gaaatgttcc cnaaaccaag gttggctccc ccaggctncc      600
tcttaccgaa ggcgcctgggc cnotttncaa ggttggggga accnaaatct tcncttatgc      660
ccnccncca cmtcttgng nancantttt ggaacccctc cnetttccct ttgctcnna      720
nccntncca aaaaaactn aaanctngc naaancttn acttcccccc tgaac      775

```

<210> 26

<211> 820

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> {1}...{820}

<223> n = A,T,C or G

<400> 26

```

anattantac agtctaator ttccccagag gtgtgtanag ggaacggggc ctaggggcat      60
cccaagata ncttatance acagtgtttt gaccaagaga tgcctgggac atttctgcga      120
gaaaagtggt cggctcccat caactctctc ctcccaatgc catccagagc ggggtgagtat      180
ccatcangcc ttccgttgga gggagtcang gaaacaacan accacagagc anacagacaa      240
atgatgacca tggggcggag cgagcctctt cctgnaccg ggggtgcana nganagccta      300
nctgaggggt cacaataaa acgttaaaga cnaagatnan cacctgtctc aagtgcaccc      360

```

| | | | | | | |
|------------|------------|-------------|------------|-------------|-------------|-----|
| ttcttaectg | acnaccagng | accannaaact | gongoctggg | gacagcctg | gganacageta | 420 |
| acnnagcaet | ccctgtccgc | cccatggcgg | tnogcntccc | tggtcctgsc | aagggaaagct | 480 |
| ccctgttggg | attnogggga | naccaaggga | scocccctct | ccanccttga | agggaaaaann | 540 |
| gatggaaatt | tncccttccg | gocnntcccc | tcttcttita | caogcccccct | nnactctntc | 600 |
| tcctctattt | ntccctgcac | acttttnacc | ccnnatttgc | ctctnattga | tcggannctn | 660 |
| ganattccac | tnnccgctnc | cntcnatng | aaanacnaaa | nactntctna | ccnggggggat | 720 |
| gggnnccctg | ntcatctctt | cttttctnct | ascnccnttt | ctttgctctt | cccttagatca | 780 |
| tccaaccntc | gntggccntn | ccccccnnn | tcctttncoc | | | 820 |

<210> 27

<211> 818

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(818)

<223> n = A,T,C or G

<400> 27

| | | | | | | |
|------------|------------|------------|-------------|-------------|-------------|-----|
| tctgggtgat | ggcctcttcc | tcctcagggg | ccctctgaatg | ctctggggcca | aagaatctct | 60 |
| tgtttctctt | ccgagccccc | ggagcgggtg | attcagccct | gcccaacctg | attctgatga | 120 |
| ctggcggtgc | tgtgaaggac | ccaagggggc | aatagggtac | caggggtccg | ggaggggggc | 180 |
| ctgctgagca | cttccgcgcc | tcacccctgc | cagccctctg | catgagctct | gggtgggttc | 240 |
| tcgcctccca | gggttctgct | cttccangca | agccanacaag | tggcgctggg | ccacactggc | 300 |
| ttctctctgc | ccctccctct | gctctganc | tctgtctctc | tgtcctgtgc | angcnccttg | 360 |
| gatctcagtt | tcctcctctc | anngaactct | gtttctgann | tcttcaantta | actntgantt | 420 |
| tatnacnann | tggncctgtc | tgtcnnactt | taetggggccn | gacccgggtaa | tcctctccctc | 480 |
| actccctctc | anttcnanna | accagcttnc | cntctctctc | ccntancocg | ccnggggaac | 540 |
| ctccttgcgc | antaccagng | gccnnnacgc | ccctnnctn | ggggggcchg | gtncctnenc | 600 |
| ctgntnaccc | cnctcncmgt | tnoctctctc | cnnccnccgc | ngccanattc | ncngtcccan | 660 |
| tnactctten | ngntctgnaa | ngntcncntn | tnnnnngnen | ngntnntnen | tcctctctenc | 720 |
| cnantgnang | tnnttunnec | ncngnacccc | nnbncnnann | nggnntcans | ctnccnengc | 780 |
| ccnnccccc | ngnattaagc | cctcnnctct | cgggcocnc | | | 818 |

<210> 28

<211> 731

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(731)

<223> n = A,T,C or G

<400> 28

| | | | | | | |
|------------|------------|-------------|------------|-------------|-------------|-----|
| aggaagggcg | gagggatatt | gtanggggatt | gagggatagg | agaataaang | gggaggtgtg | 60 |
| tcoccaactc | anggtgngnt | tctcttttga | angaggggtg | ngtttttann | ccnggtgggtg | 120 |
| gattnaaccc | catgttatgg | agtnaaaggc | tttnaaggat | ttttcgctct | ttatcagtat | 180 |
| ntanattctt | gtnaatcgga | aaatnatntt | tcnnccggaa | aaatntgtct | ccatcccgnaa | 240 |
| cttctctccg | ggtagtgcac | tttngggggc | cngccangtt | tcocaggctg | ctanaaatctt | 300 |
| actaaagntt | naagtgggan | tncaaatgaa | aacctnncac | agagnatccn | tcocccagctg | 360 |
| taantntcct | tcgcccctnt | actctgcnag | agcccaatac | ccnnngngnet | gtcnccocngn | 420 |
| nnsgcgcnc | tgaasannnc | tcgnggctnn | gancatcang | gggtttcgca | tcnaaagenn | 480 |
| ogtttencat | naaggccact | tngcctcctc | caaccnctng | ccctcnncca | tttngcgcctc | 540 |
| gggtcncct | ccgctnnntg | ccctnnnnnn | ganattttnc | ccgcctnggg | naancctcct | 600 |
| gnaatgggta | gggactnttc | ttttnacenn | gnggtntact | aactcnnctc | ccgctntcct | 660 |
| tctnaccccc | cccccctttt | caatccocanc | ggcnaatggg | gtctccocnn | cgangggggg | 720 |

nnnnccannn c

731

<210> 29
 <211> 822
 <212> DNA
 <213> Homo sapien
 <220>
 <221> misc_feature
 <222> (1)...(822)
 <223> n = A,T,C or G

<400> 29
 astagtccag tgtgggtggaa ttccatgttg ttggggcnnc ttctatgant antnttagat 60
 cgtccanacc tcacancctc ccnaccnang ctataangaa nannastaga nctgtncmct 120
 atntntacnc testannoct cennaccacc tccctcttaa cccctactgt gccctatngcm 180
 tnnctantct ntgcgcgcctn cnanccaccn gtgggcnac cncnngnatt ctcnatctcc 240
 tencsaltn gctananta ngtnacatcc ctataacctac nccaatgcta nnnctaanen 300
 tccatnantt annntaacta ccactgaent ngactttcnc atnancictc aatttgaaatc 360
 tactctgact cccacngcct annnattagc ancttcccc nccnctntct caaccacatc 420
 ntcaacaccc tatctancct ttcnccsacc nttnccctccg atcccccnnac sacccccttc 480
 ccaaatcccc nccacctgac ncttaaccnn caccatcccg gcaagccnnc ggcattttan 540
 cccatggaat cactnctngga naaaaaaac ccnaactctc tancnccnat ctccctaana 600
 aatnctcctn naattitaent noantnecat caancccaen tgaacccnaa cccctgtttt 660
 taatccctt otttccgaaa ccnacccttt annncccsac ctttngggcc ccccccctac 720
 ccaaatgaag gncncccaat cnaagaaag nccntgaaaa anccaggcna anannntccg 780
 ccaatccat cccctanttn ggggnccctt nccnngggcc cc 822

<210> 30
 <211> 787
 <212> DNA
 <213> Homo sapien
 <220>
 <221> misc_feature
 <222> (1)...(787)
 <223> n = A,T,C or G

<400> 30
 cggcgcgcgt ctctggcaca tgcctcctga atggcatcaa aagtgatgga ctgcccaattg 60
 ctagagaaaga cctctctctcc tactgtcatt atggagccct gcagactgag ggctccctct 120
 gtctgcagga ttgtatgtct gaagtgttg agtgtgctt ggagctccct atctacatna 180
 gctgggaagc ctggaggggcc tctctcgcca gctccccct tctctccacg ctctccangg 240
 acacagggg ctccaggcag cccattatct ccagnangac atggtgttct tccacgcgga 300
 cccatggggc ctgnaaggcc aggtctctct ttgacacat ctctcccgct ctgcctggga 360
 ggcctggga tccactantt ctanaacggc cgcacccncc gggggagctc cagctttttg 420
 tcccttaat gaaggttaat tgcncgtctg gctaatcat nggtcaaac nttttccctg 480
 gtgaattgt tntnccctc nonattccnc nonacatacn aacnccgaan cataaagtgt 540
 taagccctgt gggtnccctn nngaatnaac tnaactcaat taattgcctt ggctcatggc 600
 cagctttccn ttccnggaaa ctgtctntcc ctgctnttnt gaatcgccca cccccccggg 660
 aasagcgtt tgcnttttng ggggntcctt ccncttccc cctcctaan cctnccgct 720
 cgtgtgttnc nggtngcggg gaangggnat nantccccc naagggggng agnngntat 780
 ccccaaa 787

<210> 31
 <211> 799
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(799)
 <223> n = A,T,C or G

<400> 31
 tttttttttt ttttttttgc gatgctaactg ttttaattgca gggaggtgggg gtgtgtgtac 60
 calgtaccag ggcattataga agcaagaagg aaggaggggg ggcagagcgc cctgctgagc 120
 aacaagaagg tcccgacagc ttctctgtct gtctcttggc gcaggcacat gggagggcct 180
 ccgcaggggt gggggccacc agtcacagggc tgggagcact acanaggggt ggagtggtgtg 240
 gtggctggtn caaatggcct gncacanato cctacgattc ttgacacctg gatttcacca 300
 ggggaccttc tgtttcccca ngnnaacttc nttnatctcn aaagaacaca actgtttctt 360
 cngcatttct ggcctgttcat ygsaagcaca ggtgtccnat ttnggctggg acttgggtaca 420
 tatggttcog gccacacctc cccntcnaaa aagtaattca cccccccccc cmtctnltg 480
 cctggggccct taantaacca ccccggaact canttanta ttcatcttng gntgggcttg 540
 ntnatnccn cctgaangcg ccaagttgaa aggccacgoc gtnccnctc cccatagnan 600
 ntttttncnt cacttaatgc cccccccggc aacnatccaa kccccccccc tggggggccc 660
 agcccaangc ccccgntctg ggnnnccngn cncgnantcc ccagntctc ccantcngc 720
 cccnngcncc cccgcacgca gaacanaagg atngagccnc cgcannnnn aggttncnac 780
 ctgcgcccc cccnccngg

<210> 32
 <211> 789
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(789)
 <223> n = A,T,C or G

<400> 32
 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 60
 ttttnccnag ggcaggttta ttgacaaact cncgggaac aanoaggctg gggacaggac 120
 ggcaacaggc tccggcgggc gggcgggggc cctcaactgc ggtacaaaat ctgcagcccd 180
 cgtcccgct tgahtttcct ctgcagctgc aggatgcnt asaacaggc ctgggcnctn 240
 ggtgggcaac ctgggatttn aaatttccag ggcacaaatg ggtgcacncc cctcaaccac 300
 nettaggaaat agtggtntta cccnccnong ttggcncact cccnttgaa accactntc 360
 gggcttcogc catctgtct taaaacttgc aaacnctggg gccctctttt tggttantnt 420
 nccngccaca atcatnactc agactggenc gggctggccc caaaaaancc ccccaaaacc 480
 ggnccatgtc ttncggggc ttgtgcnaht tncatcaact cccgggcnca nccggnoaac 540
 ccaaaagtgc ttngggcccn caaaaaanct cccggggggc ccagtttcaa caaagtcact 600
 ccccttggcc cccaaatcct ccccccgnt ntgggggttg ggaacccaag cctctnnctt 660
 tggngggcaa gntggntccc ccttggggcc cccggtgggc cccnctctaa agaaaacncc 720
 ntccatnnca ccatccccc nngmccgnc tancangna tccctttttt taaaaaggg 780
 ccccccccg

<210> 33
 <211> 793
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(793)
 <223> n = A,T,C or G